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Society for Research on Biological Rhythms
(Conference Grant)

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Fred W. Turek
President (SRBR)

Society for Research on Biological Rhythms
Northwestern University
2153 Sheridan Road
Evanston, IL 60208

AFOSR-TR- 93 0345

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MAY 2 5 1993
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AFOSR/NL
Building 410
Bolling AFB, DC 20332-6448

6 copies of Conference Program and Abstract Book included with the final report

Approved for public release
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See reverse side for Abstract.

93-11476

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Biological Rhythms, Sleep, Circadian, Seasonal

1

13. ABSTRACT

SUMMARY OF THIRD MEETING OF THE SOCIETY FOR RESEARCH ON BIOLOGICAL RHYTHMS

From May 6-10, 1992, the Society for Research on Biological Rhythms held its third meeting at Amelia Island Plantation, Florida. The Society was formed in 1987 to promote the advancement of basic and applied research in all aspects of biological rhythms, to disseminate important research results concerning biological rhythms to the general public, to develop and enhance the eduction and training of students and researchers in the field and to foster interdisciplinary communication. This third meeting was successful in meeting the goals of the Society, particularly in the area of interdisciplinary communication.

Researchers in the field of Biological Rhythms tend to be fragmented into many disciplines and are often divided along many different lines. One way of dividing the field is along frequency lines; while some workers study biological rhythms with a period of msec, others are interested in rhythms with periods in the range of minutes, hours (i.e. ultradian or pulsatile), a day (i.e. circadian) or a year (i.e. seasonal or circannual). The field is also divided along the lines of the major disciplines within biology since rhythm biologists can be either biochemists, molecular/cellular biologists, system physiologists, behaviorists and/or ecologists. In addition, while many workers study the basic biological mechanisms involved in generating rhythmicity, others are interested in the clinical applications of a better understanding of biological rhythmicity. Even within the clinical field, researchers fall into many traditional categories including psychiatry, endocrinology, neurology, oncology, cardiology and reproduction.

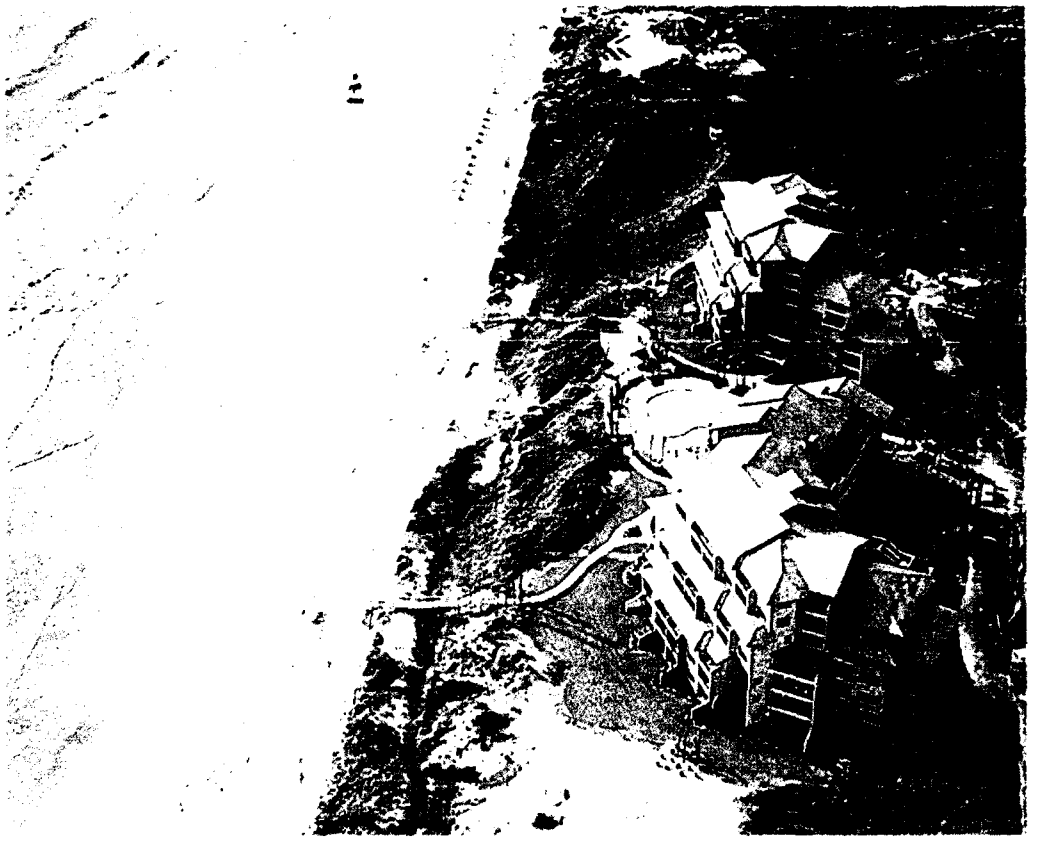
This third meeting promoted the interaction of workers in the various areas in a variety of different ways. First, there was a mixture of Symposia as well as slide and poster sessions on clinical and basic research topics. The Symposia were organized to insure that the entire frequency range of biological rhythms would be presented. While some Workshops were organized to bring together researchers in a limited fast moving field, others were organized to bridge different areas and to bring together people who normally never communicate with each other. In addition to providing a forum for interdisciplinary communication, this third meeting also brought together workers in various "hot areas" of biological rhythms.

The Society is now well established with over 500 members from 20 countries. The first two meetings of the Society were held in 1988 and 1990 and both meetings represented an exciting new forum for bringing together diverse groups of investigators together. These meetings were particularly successful in fostering interdisciplinary communication.

Second Meeting

Society for Research on

Biological Rhythms



May 9-13, 1990
Amelia Island Plantation
and Conference Center
Jacksonville, Florida

Society for Research on Biological Rhythms

Gilmer Hall
University of Virginia
Charlottesville, Virginia
U.S.A. 22901

Journal of Biological Rhythms

Program and Abstracts

for the
Second Meeting
of the
Society for Research on

Biological Rhythms

Amelia Island Plantation Conference Center
Jacksonville, Florida
May 9-13, 1990

The SRBR wishes to thank the following for their contributions:

Mini-Mitter Co., Inc.

NASA

National Institute of Mental Health

National Science Foundation

The Upjohn Company

United States Air Force,
Air Force Office of Scientific Research

Human Technologies Inc.

Table of Contents

General Information

President's Welcome

Welcome to the second meeting of the Society for Research on Biological Rhythms. The Society was formed in 1987 to promote the advancement of basic and applied research in all aspects of biological rhythms, to disseminate important research results concerning biological rhythms to the general public, to develop and enhance the education and training of students and researchers in the field and to foster interdisciplinary communication. One measure of the rapid development of our young society is the more than 50% increase in the number of registered participants and submitted abstracts for our second meeting compared with the successful inaugural meeting held two years ago in Wild Dunes, South Carolina. Over the last few years, there has been remarkable progress in understanding the basic physiological, cellular and molecular mechanisms underlying biological rhythmicity. In addition, the importance of biological rhythms for human health and disease has been made more apparent by new rigorous clinical studies. After examining the scientific program, I am sure you will agree that this second meeting offers an exciting forum for the presentation and discussion of new and important findings in the field of biological rhythms.

Fred W. Turek

Fred W. Turek
President, SRBR

Registration

Registration will take place at the Executive Conference Center Lobby at the Amelia Island Plantation, 12:00-18:00, Wednesday, May 9. For those arriving before check-in time, SRBR has reserved a hospitality suite where luggage can be left temporarily.

Registration by February 1, 1990:	
Regular SRBR Members	$100.00
Non-SRBR Members	$150.00
Student Members	$ 50.00
Student Non-members	$ 75.00
Registration after February 1, 1990:	
Regular SRBR Members	$125.00
Non-SRBR Members	$175.00
Student Members	$ 75.00
Student Non-members	$100.00
Guest Registration:	
Registration	$ 25.00
Banquet	$ 35.00

Student rates apply to registrants who will not have received the Ph.D. or M.D. degree at the time of the meeting. Registration fees include costs for opening reception and banquet dinner, as well as use of the shuttle service on Amelia Island property.

SRBR Information and Message Desk

The Society will maintain an information desk in the Patio Executive Conference Center from 8:00 am to 1:00 pm, and from 4:30 to 6:30 on May 10-12. Late arrivals can register during these times. A message board will be located next to the information desk. Check this board for mail, notes, and telephone messages. All villas are provided with telephones and a list of room locations and phone numbers of all meeting registrants will be available at the information desk.

Shuttle Service

Amelia offers an on-property shuttle service at no extra charge. Shuttles have been arranged to pick up participants 15 minutes prior to every session until the beginning of and at the close of every session, as well as social events. During these times the shuttle buses will make several tours of the grounds. Anyone wishing transportation should wait by the roadside and the bus will stop by to pick you up. If you wish shuttle service at other times, dial 5244; if you phone for shuttle service, you must wait outside for the bus. The driver will not knock on your door. A brief wait of up to 20 minutes may be needed.

Limousine Service

To the Airport: It is requested that reservations be made one day in advance of departure date; at the absolute minimum, calling **five to six hours** before flight time is necessary. Dial 5259 for "AAA Limo."

Where to Eat

Amelia has 3 major restaurants available designed to meet the requirements of any palate. *The DuneSide*, Amelia's flagship restaurant, features 5-course gourmet dining. Reservations are required, as well as jackets for gentlemen. *The Beach Club* offers casual fare; no reservations or special dress required. *The Verandah*, Amelia's seafood restaurant, is informally-styled and located in Racquet Park. For more information, and for other restaurant locations, check your "Passport" guide.

Amelia's Village Store supplies most general store items. If you wish to take the 15 minute trip to Fernandina Beach, a variety of grocery and other stores are located there.

Amelia's Facilities

The whole gamut of activities, tennis, horseback riding, sailing excursions, fishing, golf and more are available at Amelia. Check your "Passport" guide for more information.

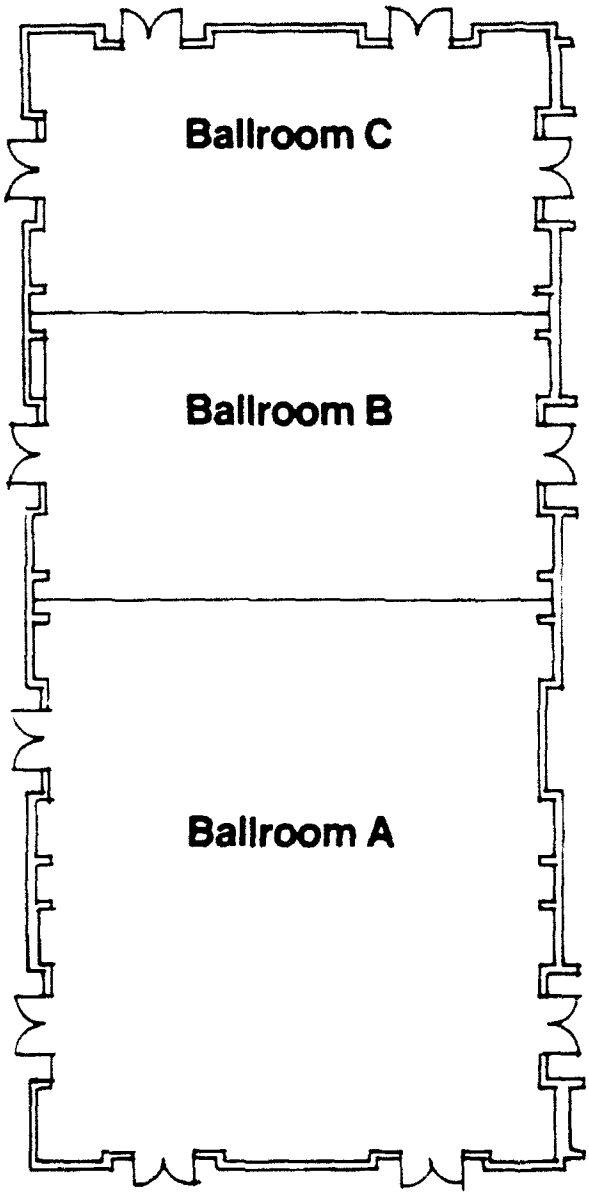
Wheels 'n Keels has a supply of rental vehicles: bicycles, paddle-boats, island hoppers, and automobiles. Call 5427.

Programs for children are available by calling 5210, and baby-sitting services, 5427.

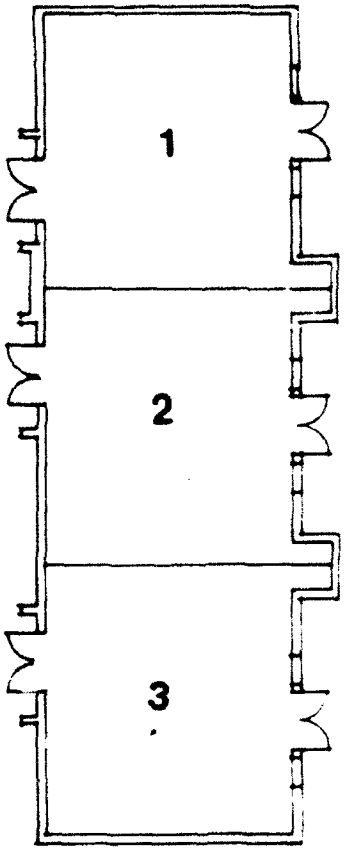
Emergency Phone Numbers

Emergency	911
Public Safety	5242 or 5243
Fire Department	261-5732
Sheriff	225-5174
General Hospital	261-3627

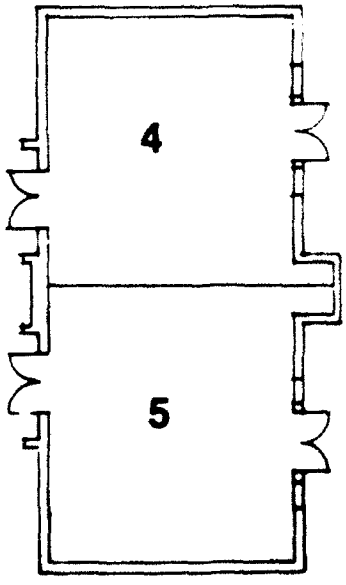
Executive Meeting Center



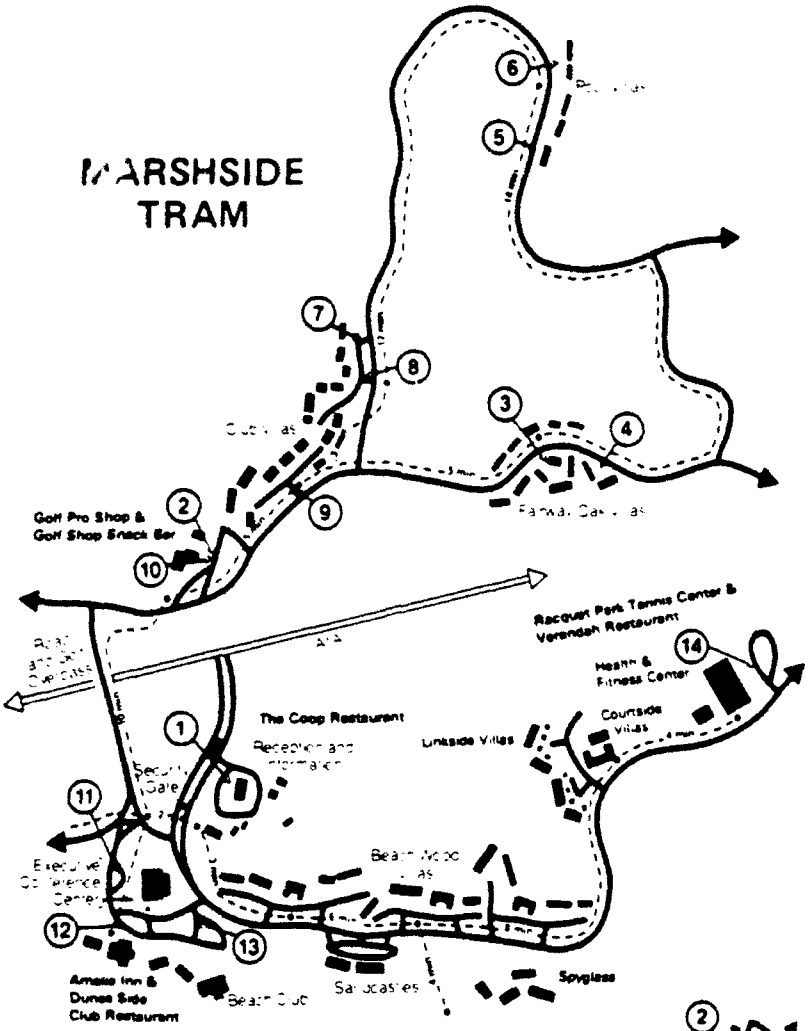
Seminar Rooms 1-3



Seminar Rooms 4 and 5



TRAM SCHEDULE



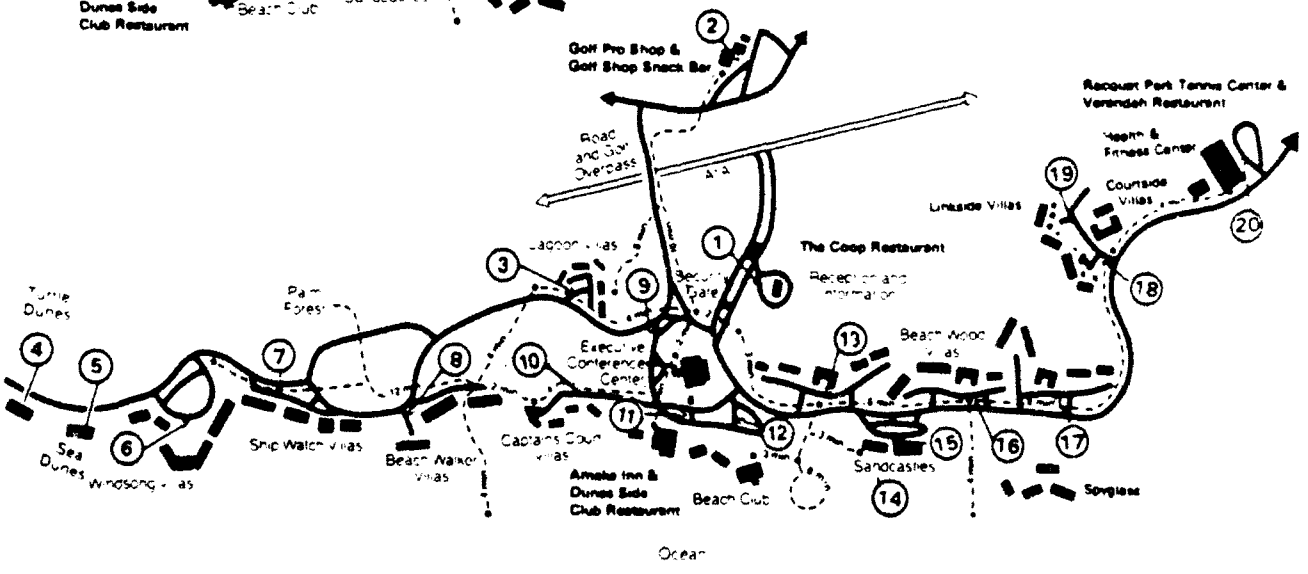
The Amelia Island Transportation trams provide complimentary transportation around the Plantation on a daily basis from 6:15 a.m. to 1:30 a.m. Tram stops are designated by signs.

Trams also provide daily transportation to the Long Point Golf Course on an on-call basis.

Trams stop only at the signs. Please allow yourself enough time to reach the stop in the area where you wish to catch the tram. Each tram stops every 15-20 minutes at designated locations.

For transportation between 1:30 p.m. and 2:00 a.m. or more information, call 5244.

Because trams operate on a predetermined route, unscheduled stops are not permitted.



Major Facilities

1 **Amelia Village**
Coop Snack Bar, Convenience Store, Wheels 'N Keels, Plantation Greenhouse, Amelia Shops

2 **Reception Center**
Front Desk, Real Estate Office and Exhibit Area, Transportation, Administrative Offices

3 **Executive Meeting Center**
Conference Services, Meeting Space

4 **Amelia Island Inn**
Dune Side Club, Admiral's Lounge, Cumberland Room, Owner's Room, Inn Suites, Leeward and Windward Rooms

5 **Beach Club**
Electronic Arcade, Amelia Angler, Youth Recreation, Beach Club Galley Lounge, Beach Services

6 **Racquet Park**
Verandah Restaurant, Tennis Pro Shop, Health and Fitness Center, Courtside Club, Racquet Park Conference Center

7 **Amelia Links**
Golf Course, Golf Pro Shop, Driving Range, Putting Green, Snack Bar

8 **Walker's Landing**
Cookout Area, Boat Ramp, Docks, Indian Burial Mound

9 **Drummond Point Park**
Basketball Court, Kids' Play Area, Nature Trail

10 **Heron Cove**
Cookout Area

11 **Willow Conservancy**

12 **Aury Island**
Kids' Play Area

13 **Long Point**
Golf Course, Golf Pro Shop, Driving Range, Putting Green, Snack Bar, 1.5 miles south of the entrance to Amelia Island Plantation, 18 holes

Dining and Entertainment Facilities

A Dune Side Club and Admiral's Lounge
Amelia Island Inn

B Beach Club Bar, Lounge and Pool Bar
Beach Club

C The Coop
Across from the Reception Center

D Amelia Links Golf Carts & Shop
Amelia Links Golf Shop

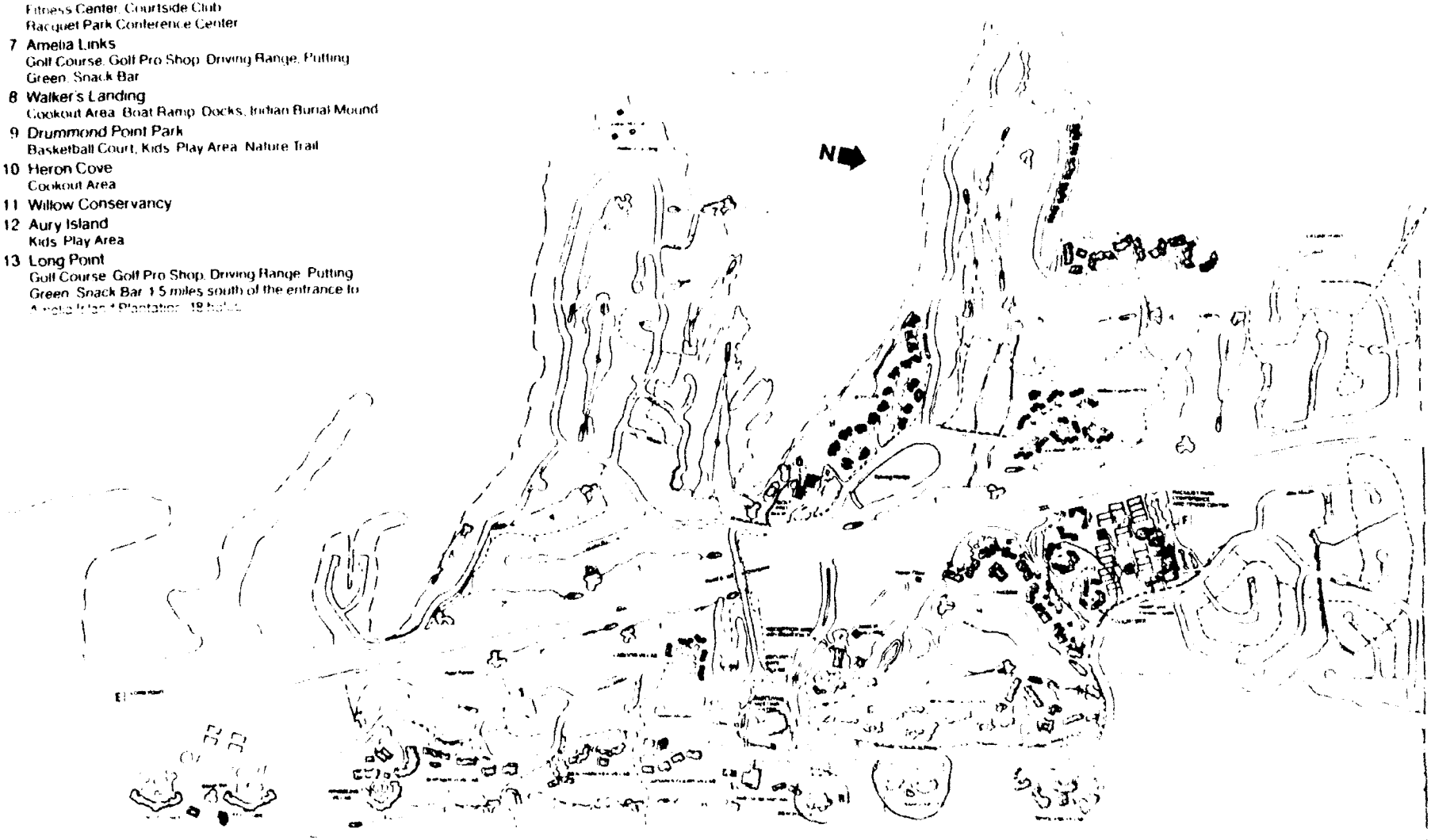
E The Putter Club at Long Point
Long Point Golf Shop

F Verandah Restaurant
Racquet Park

Bike Trails and Foot Paths

0 200 400 800

Amelia Island Plantation



Program Schedule

WEDNESDAY, MAY 9

12:00-18:00 Registration: Conference Center Patio
18:00-21:00 Opening Reception: Beach Club Deck/Pool

THURSDAY, MAY 10

8:00-10:00 ***Symposia 1 & 2***
1. Models of Regulation of Sleep, Alertness and Circadian Rhythms in Humans
Conference Room A
2. Cellular Mechanisms of Melatonin Regulation and Action
Conference Rooms B/C

10:00-10:30 Coffee Break:
Conference Center Patio

10:30-12:30 ***Workshops 1-4***
1. Molluscan Retinas: Role of Proteins in the Pacemaker System
Conference Room A
2. Feedback Effects of Activity-Rest Cycle on Circadian Clock
Conference Room B
3. Interval Timing
Conference Room C
4. Temporal Synchrony of Rhythms: Methods of Analysis
Conference Room 4 & 5

12:30-15:30 Break: Put up posters (Group A)
Conference Room 1-3

15:30-17:30 ***Slide Sessions 1-4***
1. Circadian Rhythmicity and Mental Illness
Conference Room A
2. Circadian Rhythms and Metabolism
Conference Room B
3. Entrainment Mechanisms
Conference Room C
4. Molecular and Cellular Studies of Circadian Rhythmicity
Conference Room 4 & 5

17:30-19:00 Break

19:00-20:00 ***Plenary Lecture:***
"Almost Forgotten Protocols: Mood and Activity of Humans in Temporal Isolation"
J. Aschoff
Introduced by C.S. Pittendrigh
Conference Room A-C

20:00-22:00 ***Poster Presentations, Group A***
Conference Room 1-3

FRIDAY, May 11

8:00-10:00 ***Symposia 3 & 4***
3. Interaction Between Feeding and Circadian Rhythmicity
Conference Room A
4. Regulation of Cell Cycles and Developmental Timing
Conference Room B/C

10:00-10:30 Coffee Break: Conference Center Patio

FRIDAY, MAY 11

10:30-12:30 ***Slide Sessions 5-7***
5. Circadian Rhythms in Aging
Conference Room A
6. Photic Control of Human Rhythms
Conference Room B
7. Suprachiasmatic Nucleus
Conference Room C

12:30-16:30 Break: Take down Group A posters by 14:00
Put up Group B posters after 15:00

16:30-18:30 ***Workshops 5-8***
5. Oncogenes and Entrainment
Conference Room A
6. The Eye as a Clock
Conference Room B
7. Rhythms and Aging: Human and Animal Studies
Conference Room C
8. SCN Transplants Revisited
Conference Room 4 & 5

18:30-19:00 Break

19:00-20:00 ***Business Meeting:*** Conference Room A

20:00 ***Tropical Island Banquet***
Beach Club Deck/Pool

SATURDAY, MAY 12

8:00-10:00 ***Symposia 5 and 6***
5. Circadian Mutants
Conference Room A
6. Seasonality, Photoperiodism and Reproduction
Conference Room B/C

10:00-10:30 Coffee Break: Conference Center Patio

10:30-12:30 ***Slide Sessions 8-10***
8. Circadian Timing and Sleep in Man
Conference Room A
9. Photic and Non-photic Manipulation of the Clock
Conference Room B
10. Photoperiodic and Seasonal Effects
Conference Room C

12:30-16:00 Break

16:00-18:00 ***Workshops 9-12***
9. Maternal-Fetal Communication of Photoperiodic Information
Conference Room A
10. Applications of Chronobiology to Cancer Medicine
Conference Room B
11. Anatomy of Mammalian Circadian Rhythm Regulation
Conference Room C
12. Modeling of Invertebrate Oscillator Networks
Conference Room 4-5

18:00-20:00 ***Poster Presentations — Group B***
Conference Room 1-3

Scientific Program

Thursday May 10

8:00-10:00 Room A

Symposium 1:
Models of Regulation of Sleep, Alertness and Circadian Rhythms in Humans
Alexander A. Borbely (Organizer)
Richard E. Kronauer, Robert W. McCarley

8:00-10:00 Room B/C

Symposium 2:
Cellular Mechanisms of Melatonin Regulation and Action
Martin Zatz (Organizer)
Davis C. Klein, Steven M. Reppert, Joseph S. Takahashi

10:30-12:30 Room A

Workshop 1:
Molluscan Retinas: Role of Proteins in the Pacemaker System
Gene Block (Leader)
Jon Jacklet, Felix Strumwasser, Michael Roberts, Arnold Eskin

10:30-12:30 Room B

Workshop 2:
Feedback Effects of Activity-Rest Cycle on Circadian Clock
Fred W. Turek (Leader)
Dale Edgar, Benjamin Rusak, Günther Fleissner, Olivier Van Reeth, Carmen Wickland

10:30-12:30 Room C

Workshop 3:
Interval Timing
Russel M. Church (Leader)
Jürgen Aschoff, John Gibbon, Rae Silver

10:30-12:30 Room 4 & 5

Workshop 4:
Temporal Synchrony of Rhythms: Methods of Analysis
George R. Merriam (Leader)
Michael L. Johnson, Richard E. Kronauer, Eve Van Cauter, Johannes Veldhuis, Kenneth W. Wachter, Morton B. Brown, Thomas Wagner

15:30-17:30 Room A

Slide Session 1
Circadian Rhythmicity and Mental Illness
Chairperson: Timothy Monk

15:30
1 LITHIUM ALTERS THE CIRCADIAN RHYTHM OF DISK-SHEDDING AND ENHANCES LIGHT EFFECTS IN RAT RETINAL PHOTORECEPTORS. Ch. Remé, U. Braschler, and K. Munz. University Eye Clinic, Zurich, Switzerland.

15:45
2 COMPARATIVE STUDY OF ULTRADIAN AND CIRCADIAN BIORHYTHMS OF NORMAL RATS AND RATS WITH EXPERIMENTAL NEUROSIS. J. Drescher, K. Wicht, G. Haupt, R. Weigel, A. Tsikadse, H. Weissleder. Institut of Pathophysiology/Charite, Humboldt-University Berlin Ziegelstrasse 5-9, Berlin 1 0 4 0, GDR.

16:00
3 A GENETIC ANIMAL MODEL FOR STUDIES OF DEPRESSION AND DISTURBED CIRCADIAN RHYTHMS. Gail Orpen and Meir Steiner. Neurobiology Laboratory, St. Joseph's Hospital Research Institute, and Department of Psychiatry, McMaster University, Hamilton, Ontario, Canada.

16:15
4 EFFECT OF IMIPRAMINE, AMITRIPTYLINE AND DIAZEPAM ON CIRCADIAN RHYTHMS IN THE FIELD MOUSE *Mus booduga*. P. Subramanian. Department of Animal Behaviour, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021 INDIA.

16:30
5 ALTERED WAVEFORM OF PLASMA NOCTURNAL MELATONIN SECRETION IN PREMENSTRUAL DEPRESSION. B.L. Parry, S.L. Berga, G.A. Laughlin, M.R. Klauber, S.S.C. Yen, D.F. Kripke, J.C. Gillin. Department of Psychiatry, UCSD T-004, La Jolla, CA 92093.

16:45
6 EFFECTS OF WARM AND COOL AMBIENT TEMPERATURES ON DEPRESSED PATIENTS' BODY TEMPERATURE, HORMONES AND MOOD DURING SLEEP DEPRIVATION IN A CONSTANT ROUTINE. Thomas A. Wehr, Siegfried Kasper, Jean-Robert Joseph-Vanderpool, Daniel Oren, Douglass Moul. Clinical Psychobiology Branch, Intramural Research Program, NIMH, Bethesda, MD.

17:00

7 24-HOUR CORTISOL SECRETORY PATTERNS IN DEPRESSED ADOLESCENTS. R. Dahl, N. Ryan, J. Perel, J. Puig-Antich, V. Meyer, B. Nelson. Western Psychiatric Institute of Pittsburgh, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Pittsburgh, PA.

17:15

8 SEROTONERGIC EFFECTS ON CORTISOL SECRETORY PATTERNS IN DEPRESSED CHILDREN. B. Birmaher, R. Dahl, N. Ryan, J. Perel, J. Puig-Antich. Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Pittsburgh, PA 15213.

15:30-17:30 Room B

Slide Session 2

Circadian Rhythms and Metabolism

Chairperson: Kenneth S. Polonsky

15:30

9 A MATHEMATICAL MODEL FOR THE INSULIN-GLUCOSE FEEDBACK MECHANISM ACCOUNTS FOR THE EXISTENCE OF ULTRADIAN OSCILLATIONS OF HUMAN INSULIN SECRETION. J. Sturis, K.S. Polonsky, E. Mosekilde, E. Van Cauter. Technical University of Denmark, DK-2800 Lyngby, Denmark and Dept. of Medicine, University of Chicago, IL 60637, USA.

15:45

10 ENTRAINMENT OF ULTRADIAN OSCILLATIONS OF HUMAN GLUCOSE AND INSULIN BY OSCILLATORY GLUCOSE INFUSIONS. K.S. Polonsky, J. Sturis, J.D. Blackman, E. Van Cauter. Dept. of Medicine, University of Chicago, IL 60637, USA and Technical University of Denmark, DK-2800 Lyngby, Denmark.

16:00

11 DIETARY FIBER DOES NOT MODIFY PLASMA GLUCOSE AND INSULIN PROFILES IN NORMAL AND DIABETIC SUBJECTS UNDER CONTINUOUS ENTERAL NUTRITION. C. Simon, M. Follenius, G. Brandenberger, J.L. Schlienger. Laboratoire de Physiologie et de Psychologie Environnementales, CNRS/INRS, 21 rue Becquerel, 67087 Strasbourg Cedex, France.

16:15

12 OBESITY, HYPERINSULINEMIA AND INSULIN RESISTANCE ARE DIMINISHED BY RESETTING CIRCADIAN NEUROENDOCRINE RHYTHMS. A.H. Meier and A.H. Cincotta. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

16:30

13 RESTRICTED FEEDING: MASKING OR/AND ENTRAINMENT? B. Jilge, S. Rest, H. Stahle. University of Ulm POB 4066 D - 7900 Ulm/FRG.

16:45

14 DAILY RHYTHMS OF CARBOHYDRATE- AND PROTEIN-RICH FOOD CHOICE UNDER DIFFERENT PHOTOPERIODS. P. van der Velde, K. Krauchi, R. Nil and A. Wirz-Justice. Psychiatric University Clinic, CH-4025 Basel, Switzerland.

17:00

15 DISRUPTION OF CIRCADIAN RHYTHMICITY IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY. P.C. Zee, R. Mehta, A.T. Blei and F.W. Turek. Depts. of Neurology, Medicine and Neurobiology and Physiology, Northwestern University, Chicago and Evanston, IL.

17:15

16 CIRCADIAN BODY TEMPERATURE (T_b) RHYTHMS PERSIST THROUGHOUT HIBERNATION IN GOLDEN MANTLED GROUND SQUIRRELS. D.A. Grahn, J.D. Miller, C.M. Radeke, V. Houng, and H.C. Heller. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

15:30-17:30 Room C

Slide Session 3

Entrainment Mechanisms

Chairperson: Michael Terman

15:30

17 A COMPARISON OF THE PHOTIC SENSITIVITY FOR THE PHASE-SHIFTING AND ACUTE EFFECTS OF LIGHT ON THE OSCILLATION OF MELATONIN RELEASE FROM CHICK PINEAL CELLS. L.M. Robertson and J.S. Takahashi. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

15:45

18 SPECTRAL SENSITIVITY OF THE CIRCADIAN CLOCK'S RESPONSE TO LIGHT IN DJUNGARIAN HAMSTERS. Martha M. Hotz, Jill J. Milette, Joseph S. Takahashi, and Fred W. Turek. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

16:00

19 CIRCADIAN ADJUSTMENT TO A SEASONALLY-MODULATED NATURALISTIC LIGHTING ENVIRONMENT. Michael Terman, Jiuan Su Terman, & Stephen Fairhurst. Columbia University Dept. of Psychiatry and New York State Psychiatric Institute, Box 50, 722 W. 168th St., New York, NY 10032.

16:15

20 DISK-SHEDDING AND DOPAMINE RHYTHMS UNDER SIMULATED DAWN AND DUSK. R.A. Bush, Ch.E. Remé, M. Terman*, A. Malnoe**. University Eye Clinic, Zurich, Switzerland, *New York State Psychiatric Institute, New York, **Nestlé Research Center, Lausanne, Switzerland.

16:30

21 MODULATION OF MELATONIN AND UTERINE CONTRACTILE RHYTHMS BY PHOTOPERIOD IN THE PREGNANT RHESUS MACAQUE. C.A. Ducsay and S.M. Yellon. Div. Perinatal Biology, Depts. Physiology and Pediatriatrics, Loma Linda University, Loma Linda, CA 92350.

16:45

22 DO NMDA RECEPTORS MEDIATE THE EFFECTS OF LIGHT ON CIRCADIAN BEHAVIOR IN THE HAMSTER? C.S. Colwell, M. Ralph, and M. Menaker. University of Virginia, Dept. of Biology, Charlottesville, VA 22901.

17:00

23 AMPLITUDE OF CIRCADIAN PACEMAKER AND THE PHOTOPERIODIC RESPONSE. Colin S. Pittendrigh. University of Arizona, Tucson, AZ 85721.

17:15

24 DIAPAUSE INDUCTION: ARE LOW TEMPERATURES FUNCTIONALLY EQUIVALENT TO LIGHT? G.T. Wassmer, W. Cain, E.D. DeAngelo, and S.D. Skopik. School of Life and Health Sciences, U. of Delaware, Newark, DE 19716.

15:30-17:30 Room 4 & 5

Slide Session 4

Molecular and Cellular Studies of Circadian Rhythmicity

Chairperson: Thomas Kilduff

15:30

25 EFFECT OF ENPROSTIL, A PROSTAGLANDIN E_2 ANALOGUE, ON THE CIRCADIAN RHYTHM IN DNA SYNTHESIS IN MOUSE GUT. N.H. Rubin, P.R. Laraby, J.B. Field, P.L. Rayford*, C.M. Townsend, Jr., J.C. Thompson. Department of Surgery, The University of Texas Medical Branch, Galveston, Texas, and *Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AK.

15:45

26 LIGHT INDUCES C-FOS mRNA IN THE SUPRACHIASMATIC NUCLEUS OF HAMSTER. Jon M. Kornhauser, Dwight E. Nelson, Kelly E. Mayo, and Joseph S. Takahashi. Department of Biochemistry, Molecular Biology and Cell Biology, and Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

16:00

27 PHASE DEPENDENT INDUCTION OF THE PROTO-ONCOGENE fos (c-fos) IN HAMSTER SUPRACHIASMATIC NUCLEUS (SCN). J. Serviere, G. Gendrot, D. Menetrey*, F. Xavier and J. de Pommery*. INRA 78350 Jouy en Josas, *INSERM U161 Paris, France.

16:15

28 MELATONIN INDUCES c-FOS EXPRESSION IN THE SUPRACHIASMATIC NUCLEI. T.S. Kilduff, H.B. Landel, G.S. Nagy, H.C. Heller, and W.C. Dement. Depts. of Psychiatry and Biological Sciences. Stanford University, Stanford, CA 94305.

16:30

29 CHARACTERIZATION OF MELATONIN RECEPTORS IN THE RAT SUPRACHIASMATIC NUCLEI AND AREA POSTREMA: AFFINITY SHIFTS WITH GUANINE NUCLEOTIDES AND MONOVALENT CATIONS. Jarmo T. Laitinen and Juan M. Saavedra. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

16:45

30 THE AMPLIFICATION OF *DROSOPHILA per* GENE HOMOLOGS FROM RAT AND MOUSE BRAIN cDNA LIBRARIES. Thomas Maler, Brian F. O'Dowd, Russell Van Gelder, and Harvey Moldofsky. Dept. of Psychiatry, Toronto Western Hospital, Addicition Research Foundation, Ontario, Dept. of Psychiatry, Stanford University School of Medicine, Dept. of Clinical Biochemistry, University of Toronto.

17:00

31 CYCLIC AMP, CELL DIVISION CYCLES, AND CIRCADIAN OSCILLATORS IN *EUGLENA*. Leland N. Edmunds and Isabelle A. Carré. Division of Biological Sciences, State University of New York, Stony Brook, NY 11794.

17:15

32 MONOCLONAL ANTIBODIES TO A UNIQUE ANTIGEN IN NEUROSECRETORY CELLS OF A CIRCADIAN ORGAN, THE *APLYSIA* EYE. Rachel L. Cox, Alan M. Kuzirian, Mark A. Scanzillo, David L. Glick, Daniel P. Viele, and Felix Strumwasser. Laboratory of Neuroendocrinology, Marine Biological Laboratory, Woods Hole, MA 02543.

19:00-20:00 Room A-C

Plenary lecture

"Almost Forgotten Protocols: Mood and Activity of Humans in Temporal Isolation"

J. Aschoff

Introduced by C.S. Pittendrigh

20:00-22:00 Room 1-3

Poster Presentations, Group A

Human Rhythms

33 LOCOMOTOR ACTIVITY ACCELERATES RE-ENTRAINMENT OF THE CIRCADIAN TEMPERATURE RHYTHM TO A DELAYED SLEEP-WAKE CYCLE IN MAN. K.P. Schmidt, W.K. Koehler, B. Pflug. Zentrum der Psychiatrie, Universitätsklinik Frankfurt, Frankfurt/Main, West-Germany.

34 PHASE-SHIFTING EFFECT OF INDIRECT (CEILING-MOUNTED) BRIGHT LIGHT EXPOSURE ON THE HUMAN CIRCADIAN PACEMAKER. Jeanne F. Duffy, Theresa L. Shanahan, and Charles A. Czeisler. Center for Circadian and Sleep Disorders Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115.

35 INTERPRETATION OF THE CIRCADIAN PATTERN OF MELATONIN SERUM CONCENTRATIONS IN WOMEN: WHICH METHOD IS BEST? Sarah L. Berga, Gail A. Laughlin, Michael L. Johnson, Anne B. Loucks and Samuel S.C. Yen. The University of Pittsburgh, The University of Virginia, and the University of California, San Diego, CA.

36 EFFECT OF PHOTOTHERAPY ON MOOD DISORDERS. Tetsushi Tsujimoto, Koichi Hanada, Toshiki Shioiri, Kazushi Daimon, Takayuki Kitamura and Saburo Takahashi. Department of Psychiatry, Shiga University of Medical Science, Seta Tsukinowacho, Otsu, Shiga, Japan 520-21.

37 ULTRADIAN RHYTHMS IN EVENT-RELATED CORTICAL ACTIVITY AND PERFORMANCE. Polly Stone and John Harsh, Department of Psychology, University of Southern Mississippi, Hattiesburg, MS 39406-5025.

38 DIURNAL VARIATION IN N100-P200 COMPLEX OF THE VISUAL EVOKED POTENTIAL. Nancy Jo Wesensten and Pietro Badia*. WRAIR, Washington, D.C. USA; *Bowling Green State University, OH USA.

39 RHYTHMIC ORGANIZATION OF SPONTANEOUS MOVEMENTS IN PREMATURE INFANTS LESS THAN 34 WEEKS GESTATIONAL AGE. Marie J. Hayes, Savitri P. Kumar, Lonnie Plante, and Maria Delivoria-Papadopoulos, Dept. of Pediatrics, University of Pennsylvania, and Dept. of Psychology, University of Maine.

40 A WAVEFORM-INDEPENDENT DECONVOLUTION TECHNIQUE TO ANALYZE *IN VIVO* NEUROHORMONE SECRETION. M.L. Johnson*, A.E. Lassiter*, and J.D. Veldhuis. Interdisciplinary Graduate Biophysics Program, Division of Endocrinology and Metabolism, Departments of Pharmacology and Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

41 EPISODIC RENIN RELEASE DURING SLEEP IN SOME PATHOLOGICAL CASES. G. Brandenberger, M. Follenius, J.L. Imbs, H. Schulz, J. Krieger. Laboratoire de Physiologie et de Psychologie Environnementales, CNRS/INRS, 21 rue Becquerel, 67087 Strasbourg Cedex, France.

42 THE TIMING OF CARDIAC ARRESTS. J.R. Davidson. Ontario Prehospital Care Study Group. Co-ordinating Centre: Hotel Dieu Hospital, Kingston, Canada.

Seasonal Reproduction

43 EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON EGG LAYING IN A MARINE MOLLUSC, *APLYSIA CALIFORNICA*. Nancy L. Wayne and Gene D. Block, Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

44 NOVEL COMBINATIONS OF CIRCADIAN ECLOSION RHYTHM AND PHOTOPERIODIC DIAPAUSE IN LABORATORY STRAINS OF DROSOPHILA LITTORALIS. Pekka Lankinen. Department of Genetics, University of Oulu, SF 90570 Oulu, Finland.

45 RESPONSIVENESS OF THE HAMSTER REPRODUCTIVE APPARATUS TO A SINGLE LONG DAY: IS THERE MAXIMAL SENSITIVITY AT WEANING? Cynthia M. Finley and Carol S. Whaling. Department of Psychology, University of California, Berkeley, CA 94720.

46 PHOTOPERIODIC INFLUENCES ON BEHAVIOR REQUIRE THE PINEAL IN MALE AND FEMALE SYRIAN HAMSTERS. Jonathan Karp, Michael Miernicki, and J. Bradley Powers. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

47 EFFECT OF A SIMULATED NATURAL PHOTOPERIOD CYCLE ON TIMING OF ANNUAL CYCLES IN TWO HAMSTER SPECIES. Jeffrey A. Elliott and Bruce D. Goldman. Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269-4154.

48 PROLACTIN-DEPENDENT SEASONAL CHANGES IN PELAGE: ROLE OF THE PINEAL GLAND AND DOPAMINE. Lori L. Badura and Bruce D. Goldman. Dept. Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

49 LIGHT-MEDIATED GONADAL GROWTH INDEPENDENT OF SUSTAINED PINEAL SECRETORY ACTIVITY IN SIBERIAN HAMSTERS. Carol S. Whaling[1] and Cynthia M. Finley[2]. [1]Group in Endocrinology and [2]Department of Psychology, University of California, Berkeley, CA 92720.

50 EFFECTS OF NEAR-ULTRAVIOLET RADIATION ON MELATONIN RHYTHMS AND REPRODUCTIVE DEVELOPMENT IN SIBERIAN HAMSTERS. Karen T. Stewart, John P. Hanifin, Mark D. Rollag, Milton Stetson, and George C. Brainard. Dept. of Neurology, Jefferson Medical College, Dept. of Anatomy, Uniformed Services University of Health Sciences, and School of Life and Health Sciences, University of Delaware.

51 SHORT-DAY INDUCED REGRESSION IN MALE MEADOW VOLES MAY BE REVERSED BY BRIEF EXPOSURE TO A LONG PHOTOPERIOD. Leslie R. Meek, Gretchen D. Reeves, Theresa M. Lee and John Dark. University of Michigan, Ann Arbor, MI and University of California, Berkley, CA.

52 ENDOGENOUS CIRCANNUAL RHYTHMS OF REPRODUCTION IN EWES: EFFECTS OF EXPOSURE TO CONSTANT PHOTOPERIOD AND PINEALECTOMY. D. O'Callaghan, *F.J. Karsch, M.P. Boland and J.F. Roche. University College Dublin, Ireland and *Reproductive Sciences Program, The University of Michigan, Ann Arbor, MI 48109.

53 ABOLITION OF THE SEASONAL VARIATIONS OF SEXUAL ACTIVITY IN THE HE-GOATS BY A RAPID ALTERNATION BETWEEN LONG AND SHORT DAYS. J.A. Delgadillo, B. LeBoeuf, and P. Chemineau. INRA, Physiolgie de la Reproduction, 37380 Nouzilly, France.

Effects of Activity on the Circadian Clock

54 SOCIAL ENTRAINMENT IN THREE SHREWS. M. Menaker, M.A. Vogelbaum, and N. Kassell. Division of Biomedical Engineering and Department of Biology, University of Virginia, Charlottesville, VA 22901.

55 WHEEL RUNNING ACTIVITY IS A DETERMINANT OF CIRCADIAN RHYTHM PERIOD IN THE MOUSE. Connie E. Martin, Dale M. Edgar and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94304.

56 IS THE PHASE-SHIFTING EFFECT OF TRIAZOLAM ON THE HAMSTER'S CIRCADIAN CLOCK MEDIATED BY A CHANGE IN BODY TEMPERATURE? Carmen Wickland, Fred W. Turek. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

57 RESTRAINT DOES NOT BLOCK THE PHASE SHIFTING EFFECTS OF A PROTEIN SYNTHESIS INHIBITOR ON THE HAMSTER CIRCADIAN CLOCK. Deborah A. Hinch and Fred W. Turek. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

58 CHRONIC EXERCISE HAS NO EFFECT ON SERUM MELATONIN LEVELS IN FEMALE HAMSTERS ON LONG OR SHORT PHOTOPERIOD. David R. Pieper, Catherine A. Lobocki, and Katarina T. Borer. Providence Hospital, Dept. of Physiology, Southfield, MI and The University of Michigan, Dept. of Kinesiology, Ann Arbor, MI.

59 THE ROLE OF AMINERGIC AGENTS IN CIRCADIAN TIMEKEEPING. Peter P. Sayeski, Dale M. Edgar, Joseph D. Miller, and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94305.

60 DIETARY INDUCED OBESITY INFLUENCES ANTICIPATORY ACTIVITY IN RATS. Judith E. Persons and Friedrich K. Stephan, Dept. of Psychology, Florida State University, Tallahassee, FL.

61 FREE-ACCESS TO A RUNNING WHEEL ALTERS BOTH FREE-RUNNING PERIOD AND BRAIN MONOAMINE RHYTHM IN BLINDED RATS. T. Shioiri, *K. Takahashi, K. Hanada, N. Yamada, T. Tsujimoto, K. Daimon, T. Kitamura, and S. Takahashi. Dept. of Psychiatry, Shiga Univ. Med. Sci., Otsu, *Div. Ment. Disord. Res., Nat'l. Inst. Neurosci., Kodaira, Tokyo, Japan.

62 LOCOMOTOR ACTIVITY AND LIGHT PULSES AS COMPETING ZEITGEBER STIMULI IN THE SCORPION CIRCADIAN SYSTEM. W. Hohmann, S. Michel, G. Fleissner. Zoologisches Institute der Goethe Universität Frankfurt/Main, Siesmayerstr. 70, D-6000 Frankfurt/Main, F.R.G.

Entrainment

63 HOW DOES DAYTIME LIGHT PREVENT DAMPING OF THE MELATONIN RHYTHM IN CULTURED CHICK PINEAL CELLS: REBOUND OR ENTRAINMENT? Martin Zatz. Section on Biochem. Pharm., LCB, NIMH. Bethesda, MD 20892.

64 PATTERNED LIGHT AND ENTRAINMENT OF LOCOMOTOR ACTIVITY IN MICE. B.C. Wilson, A.J.S. Summerlee and J.R. Spurgeon. Biomedical Sciences, Ontario Veterinary College, Guelph, Ontario N1G 2W1, Canada.

65 EFFECT OF MONOCHROMATIC LIGHT ON THE CHARACTERISTICS OF THE CIRCADIAN RHYTHM DURING THE ONTOGENY OF THE CRAYFISH. M.L. Fanjul-Moles[1], M. Miranda-Anaya[2], and B. Fuentes-Pardo[1]. [1]Depto. de Biología, Fac. de Ciencias; [2]Depto. de Fisiología, Fac. de Medicina, U.N.A.M., 04510, México, D.F.

66 ENTRAINMENT OF BEHAVIORAL CIRCADIAN RHYTHMS BY INFUSION OF PHYSIOLOGICAL LEVELS OF MELATONIN. C.C. Chabot and M. Menaker. Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA.

67 AMBIENT TEMPERATURE INFLUENCES ENTRAINMENT OF SIBERIAN HAMSTER ACTIVITY RHYTHMS. Elena M. Thomas. Dept. of Psychology, University of California, Berkeley, CA 94720.

68 THE ENTRAINMENT OF CIRCADIAN ACTIVITY RHYTHMS TO FEEDING SCHEDULES. H. Abe* and S. Ebihara. Dept. Aerospace Psychology, Res. Inst. Env. Med., Nagoya University and Dept. Animal Physiology, Nagoya University, Nagoya 464-01, Japan. *Present address: Dept. Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

69 THE ROLE OF THE LATERAL HYPOTHALAMIC AREA (LHA) IN THE REGULATION OF HAMSTER LOCOMOTOR RHYTHMS. N.L. Goodless-Sanchez, R.Y. Moore, and L.P. Morin. Depts. of Psychiatry and Neurology, SUNY Stony Brook, Stony Brook, NY 11794.

70 THE ROLE OF EFFERENT PATHWAYS IN PHASE-SHIFTING CIRCADIAN RHYTHMS OF WHEEL-RUNNING IN THE GOLDEN HAMSTER. M.E. Harrington and T. Rahmani, Dept. of Psychology, Smith College, Northampton, MA 01063.

71 IMITATIONS OF THE CIRCADIAN CHANGES IN RABBIT PHOTIC RESPONSES, ELICITED BY STIMULATION OF THE CERVICAL SYMPATHETIC NERVES OR SUPRACHIASMATIC NUCLEI. A.C. Bobbert, F. Eggelmeijer, and J.J. Riethoven. Dept. of Physiology and Physiological Physics, Leiden University, Leiden, The Netherlands.

Circadian and Ultradian Rhythms

72 SHORT TAU(DD) IN ALBINO MUTANT MICE: ABSENCE OF A NORMAL AFTER—EFFECT? Bernard Possidente, Carol Lyons, and Elizabeth Carlson. Biology Dept., Skidmore College, Saratoga Springs, NY 12866.

73 EFFECTS OF THYROIDECTOMY AND ORCHIDECTOMY ON CIRCADIAN RHYTHMS OF WHEEL RUNNING ACTIVITY IN RATS ON A LIGHT-DARK CYCLE. J.E. Ottenweller, W.N. Tapp, and B.H. Natelson. Neurobehavioral Unit (127A), VA Medical Center, East Orange, NJ 07019 and Dept. of Neurosciences, New Jersey Medical School, Newark, NJ.

74 CIRCADIAN RHYTHMICITY OF SERUM CORTICOSTERONE OF INTACT AND PINEALECTOMIZED MICE*. Li Jing Cai, Wang Ai Min, and Li Cheng Ji. Dept. of Physiology, Shenyang College of Pharmacy, Liaoning, China.

75 EEG DELTA ACTIVITY IN SQUIRREL MONKEY SLEEP DEPENDS ON CIRCADIAN PHASE AND LENGTH OF TIME AWAKE. E.B. Klerman, T.A. Houpt, R.E. Mistlberger, D.M. Edgar, Z. Boulos and M.C. Moore-Ede. Dept. of Physiology, Harvard Medical School, and Institute for Circadian Physiology, 677 Beacon St., Boston, MA 02215.

76 CIRCADIAN RHYTHMICITY AND REPRODUCIBILITY IN NUCLEIC ACIDS SYNTHESIS OF SEVERAL MURINE TISSUES. Wange Ai Min, Li Jing Cai, Wang Yukun, Ma Kongchen, Wang Min, Ge Shu. Dept. of Physiology, Shenyang College of Pharmacy, Liaoning, China.

77 RESEARCH ON RHYTHMS IN RABBITS. B. Jilge, H. Stähle, S. Rest. University of Ulm POB 4066 D-7900 Ulm/F.R.G.

78 TIMING OF BIRTH IN HAMSTERS. N. Viswanathan and Fred C. Davis. Dept. of Biology, Northeastern University, Boston, MA 02115.

79 TIMING OF DEVELOPMENT OF THE CIRCADIAN CLOCK CONTROLLING THE RELEASE OF SPERM FROM THE TESTIS OF A MOTH. Jadwiga M. Giebultowicz. Dept. of Zoology, University of Maryland, College Park and USDA, ARS, ICEL, Beltsville, MD 20705.

80 CIRCADIAN RHYTHM OF CHEMOTAXIS IN *CHLAMYDOMONAS*. Edward Byrne and Carl Hirschie Johnson*. Dept. of Biology, Middle Tennessee State University, Murfreesboro, TN 37132, and *Dept. of Biology, Vanderbilt University, Nashville, TN 37235.

81 STATISTICAL ANALYSIS OF ULTRADIAN RHYTHMS: A COMPARISON OF DIFFERENT METHODS OF TIME SERIES ANALYSIS. U. Siebert and F. Wollnik. Dept. of Biology, University of Konstanz, D-7750 Konstanz, F.R.G.

82 MASCULINIZATION OF FEMALE SLEEP CYCLE RHYTHMICITY BY PERINATAL INJECTION OF TESTOSTERONE. W. Fishbein, J. Fang, S.W. Yang, and S.J. Tien. Neurocognition Program. CUNY, City College and Graduate School, NY 10031.

82A DIURNAL RHYTHM INFLUENCING LEARNING IN THE MARINE FISH *Serranus scriba* CUV. Nikola S. Kovacevic and Ivan M. Milosevic. Institute for Biological and Medical Research of Montenegro, Dept. of Marine Biology, Kotor, Yugosiavia.

82B THE PINEAL, THE RETINAE AND MELATONIN IN THE CIRCADIAN SYSTEM OF THE EUROPEAN RUINS LIZARD. Augusto Foá, Daniel Janik*, Lucia Minutini. Diparimento di Scienze del Comportamento Animale e dell'Uomo, Universtá di Pisa, Italy. *Max-Planck-Institut Fuer Verhaltensphysiologie, 8138 Andechs, F.R.G.

Friday, May 11

8:00-10:00 Room A

Symposium 3

Interaction Between Feeding and Circadian Rhythmicity

Eve Van Cauter (Organizer)
Mary F. Dallman, Sarah F. Leibowitz, Kenneth S. Polonsky, Friedrich K. Stephan.

8:00-10:00 Room B & C

Symposium 4

Regulation of Cell Cycles and Developmental Timing

David R. Soll (Organizer)
Paul Russell, Rudolf Raff

10:30-12:30 Room A

Slide Session 5

Circadian Rhythms in Aging

Chairperson: Georges Copinschi

10:30

83 ALTERATIONS OF ACTIVITY RHYTHMS OF RATS RECEIVING TRANSFECTED CELL LINES. A. Morris, B. Tate-Ostroff, R.E. Majocha, and C.A. Marotta. Neurobiology Laboratory, Dept. of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

10:45

84 FREE-RUNNING RHYTHMS, THE ENDOGENOUS COMPONENT OF TEMPERATURE AND THE EFFECT OF LIGHT INTENSITY ON CIRCADIAN RHYTHMS IN YOUNG AND OLD RATS. Wil Witting, Majid Mirmiran, Nico Bos, and Dick Swaab. Netherlands Institute for Brain Research, Meibergdreef 33, NL-1105AZ Amsterdam, The Netherlands.

11:00

85 STIMULATED ACTIVITY INDUCED BY TRIAZOLAM OR DARK PULSES DOES NOT PHASE SHIFT THE CIRCADIAN CLOCK OF OLD HAMSTERS. O. Van Reeth* and F.W. Turek. Interdisciplinary Research Institute in Human and Nuclear Biology (I.R.I.B.H.N.), Université Libre de Bruxelles, Faculté de Médecine-Erasme, Brussels, Belgium, and Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208 USA.

11:15

86 CIRCADIAN PROFILES OF CORTISOL AND TSH DURING SLEEP DEPRIVATION IN ELDERLY MEN. D. Roland, J. Blackman, K.S. Polonsky, E. Van Cauter. Dept. of Medicine, University of Chicago, IL 60637.

11:30

87 CIRCADIAN RHYTHM DISORDERS OF SLEEP-WAKING, BODY TEMPERATURE AND MELATONIN SECRETION IN ELDERLY-PATIENTS WITH DEMENTIA AND THEIR PHOTOTHERAPY. M. Okawa, K. Mishima, Y. Hishikawa, S. Hozumi, H. Hori and K. Takashashi. Dept. of Neuropsychiatry, Akita University School of Medicine, Hondo 1-1-1 Akita. Kyowa Hospital, Akita and National Center of Psychiatry and Neurology, Instute of Neuroscience, Japan.

11:45

88 SLEEP IN HEALTHY 80 YEAR OLDS FOLLOWING A 6-HOUR PHASE ADVANCE IN ROUTINE. Daniel J. Buysse, Timothy H. Monk. Human Chronobiology Program, WPIC, University of Pittsburgh, Pittsburgh, PA 15213.

12:00

89 THE APPLICATION OF PHOTOTHERAPY TO SLEEP DISORDERS IN ELDERLY PATIENTS. Marie Dumont, Gary S. Richardson, and Charles A. Czeisler. Center for Circadian and Sleep Disorders Medicine. Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

10:30-12:30 Room B

Slide Session 6

Photic Control of Human Rhythms

Chairperson: Thomas Wehr

10:30

91 RAPID ADJUSTMENT TO NIGHT SHIFT USING A SINGLE PULSE OF BRIGHT LIGHT. Scott S. Campbell and Drew Dawson. Institute for Circadian Physiology, Boston, MA

10:45

92 BODY TEMPERATURE AND SLEEP/WAKE DISTRIBUTION IN BLIND SUBJECTS WITH AND WITHOUT SLEEP COMPLAINTS UNDER MINIMAL MASKING CONDITIONS. Heinz Martens, Hartman Endlich, Gunther Hildebrandt, Rudolf Mood. Institut für Arbeitsphysiologie und Rehabilitationsforschung, Philipps-Universität, Marburg, West Germany.

11:00

93 USE OF POLAR PHASE/AMPLITUDE VECTORS TO EVALUATE THE RESPONSE OF THE HUMAN CIRCADIAN SYSTEM TO LIGHT. Megan E. Jewett, Richard E. Kronauer, and Charles A. Czeisler. Center for Circadian and Sleep Disorders Medicine, Dept. of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

11:15

94 THE PHASE ADVANCING EFFECTS OF MELATONIN ADMINISTRATION IN HUMANS: EVIDENCE FOR A PHASE RESPONSE CURVE. Robert L. Sack, Alfred J. Lewy, Jeanne Latham, Mary Blood. Sleep and Mood Disorders Laboratory, Oregon Health Sciences University, Portland, OR 97210.

11:30

95 ENDOGENOUS CIRCADIAN RHYTHM OF THYROID STIMULATING HORMONE CAN BE PHASE SHIFTED BY LIGHT EXPOSURE. J.S. Allan and C.A. Czeisler. Center for Circadian and Sleep Disorders Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA.

11:45

96 ACTILLUME ASSESSMENTS OF HUMAN PHOTIC EXPOSURE. Daniel F. Kripke, Roger Cole, and William Gruen*. Dept. of Psychiatry, V-116-A, UCSD, La Jolla, CA 92093 USA, and *Ambulatory Monitoring, Inc., 731 Saw Mill River Road, Ardsley, NY 10502.

12:00

97 LIGHT-INDUCED ADAPTATION OF THE HUMAN CIRCADIAN SYSTEM TO NIGHT WORK. C.A. Czeisler, M.P. Johnson, J.F. Duffy, E.N. Brown, J.M. Ronda, R.E. Kronauer. Center for Circadian and Sleep Disorders Medicine, Boston, MA 02115.

12:15

98 LIGHT THERAPY IN SAD PATIENTS: CLINICAL RESULTS AND EFFECTS ON THE INTERNAL PHASE RELATIONSHIP BETWEEN LOCOMOTOR ACTIVITY AND BODY TEMPERATURE RHYTHM. W.K. Koehler, K.P. Schmidt, B. Pflug. Zentrum der Psychiatrie, J.W. Goethe — Universität, Heinrich-Hoffmann-Str. 10, Frankfurt am Main, F.R.G.

10:30-12:30 Room C

Slide Session 7

Suprachiasmatic Nucleus

Chairperson: W.J. Rietveld

10:30

99 SCN-ABLATION AND HAMSTER HOARDING BEHAVIOR. R. Mistlberger, C.H. Jones. Depts. Psychology, Simon Fraser University, Burnaby, BC and University of British Columbia, Vancouver, BC.

10:45

100 GABAERGIC MODULATION OF 2-DEOXYGLUCOSE UPTAKE IN THE SCN *IN VITRO*. J.D. Miller, V.H. Cao, H.C. Heller, and T.S. Kilduff. Depts. of Biological Sciences and Psychiatry, Stanford University, Stanford, CA 94305.

11:00

101 CONTROL OF THE PERIOD AND PHASE OF CIRCADIAN RHYTHMS RESTORED BY ANATOMICALLY CHARACTERIZED SUPRACHIASMATIC GRAFTS. E.L. Bittman, J. Basil, J.M. Watt, and M.N. Lehman*, Dept. of Zoology, University of Massachusetts, Amherst, MA 01003, and *Dept. of Anatomy and Cell Biology, University of Cincinnati School of Medicine, Cincinnati, OH 45267.

11:15

102 NEURAL TRANSPLANT OF CULTURED SUPRACHIASMATIC NUCLEI. J. Ding, J. Buggy, L. Terracio* and P.J. DeCoursey**. Depts. of Physiology, Anatomy*, School of Medicine and Dept. of Biological Sciences**, University of South Carolina, Columbia, SC 29208.

11:30

103 SPATIAL DISTRIBUTION AND INTERACTION OF SCN NEUROPEPTIDES IN INTACT HAMSTERS AND RATS AND IN SCN TRANSPLANTS: TOWARDS QUANTITATIVE, 3-DIMENSIONAL VISUALIZATION. J. Buggy and J.D. Ding. Dept. of Physiology, University of South Carolina School of Medicine, Columbia, SC. P.J. DeCoursey, Dept. of Biological Sciences, University of South Carolina. Columbia, SC 29208.

11:45

104 LOCALIZATION OF A CIRCADIAN PACEMAKER TO THE VENTROLATERAL SUPRACHIASMATIC NUCLEUS (SCN). M.U. Gillette and T.K. Tcheng. Dept. of Physiology and Biophysics and Neuroscience Program, University of Illinois, Urbana, IL 61801.

12:00
105 PROPERTIES OF CULTURED SUPRACHIASMATIC NUCLEUS CELLS. W.J. Rietveld, E. Marani, R.J. van den Berg, and I. Walsh. Dept. of Physiology, University of Leiden, PO Box 9604, 2300 RC Leiden, The Netherlands.

12:15
106 THE PIGEON SUPRACHIASMATIC NUCLEUS (SCN) AND INTERGENICULATE LEAFLET (IGL): A TRACT TRACING AND IMMUNOCYTOCHEMICAL STUDY. R.B. Norgren, Jr., and Michael N. Lehman. Dept. of Anatomy and Cellular Biology, University of Cincinnati College of Medicine, Cincinnati, OH.

16:30-18:30 Room A
Workshop 5
Oncogenes and Entrainment
Bejamin Rusak (Leader)
John Kornhauser, Bill Schwartz, David Earnest
Tony van den Pol

16:30-18:30 Room B
Workshop 6
The Eye as a Clock
Herbert Underwood (Leader)
Greg Cahill, Anna Wirz-Justice, Gene Block

16:30-18:30 Room C
Workshop 7
Rhythms and Aging:
Human and Animal Studies
Timothy H. Monk (Leader)
Mary Ann Brock, Melanie Kittrell, Phyllis Zee

16:30-18:30 Room 4 & 5
Workshop 8
SCN Transplants Revisited
Raul Aguilar-Roblero (Leader)
Patricia De Coursey, Martin Ralph, Fred Davis,
Rae Silver, Michel Lehman

19:00-20:00 Room A
Business Meeting

Saturday, May 12

8:00-10:00 Room A
Symposium 5
Circadian Mutants
Michael W. Young (Organizer)
Jay C. Dunlap, William Schwartz,
Michael Menaker

8:00-10:00 Room B & C
Symposium 6
Seasonality, Photoperiodism and Reproduction
Irving Zucker (Organizer)
Eric L. Bittman, Michael H. Hastings,
Kathleen D. Ryan

10:30-12:30 Room A
Slide Session 8
Circadian Timing and Sleep in Man
Chairperson: Anna Wirz-Justice

10:30
107 THE STATISTICAL ANALYSIS OF CIRCADIAN PHASE AND AMPLITUDE IN CONSTANT ROUTINE CORE TEMPERATURE DATA. E.N. Brown and C.A. Czeisler. Center for Circadian and Sleep Disorders Medicine, Division of Endocrinology and Hypertension, Brigham and Women's Hospital, Boston, MA 02115.

10:45
108 ROLES OF SLEEP AND CIRCADIAN RHYTHMICITY IN MODULATING PITUITARY-DEPENDENT SECRETIONS AND GLUCOSE REGULATION IN MAN. J.D. Blackman, D. Roland, J. Sturis, T. Marcinkowski, K.S. Polonsky, E. Van Cauter. Dept. of Medicine, University of Chicago, IL 60637.

11:00
109 CIRCADIAN TIMING OF SLEEP IN LONGHAUL FLIGHT CREWS. P.H. Gander and R.C. Graeber. Aerospace Human Factors Research Division, NASA Ames Research Center, Moffett Field, CA 94035.

11:15
110 TRIAZOLAM-INDUCED PROLACTIN (PRL) AND GROWTH HORMONE (GH) RELEASE IN NORMAL MEN SUBMITTED TO AN 8-HOUR DELAY OF THE SLEEP-WAKE CYCLE. A. Caufriez, G. Copinschi, A. Van Onderbergen. M. Szyper, C. Robyn, and E. Van Cauter. Université Libre de Bruxelles, Brussels, Belgium.

11:30
111 EFFECTS OF THE "JET LAG DIET" ON THE ADJUSTMENT TO A PHASE ADVANCE. Margaret L. Moline, Charles P. Pollak, Daniel R. Wagner, Steven Zendell, *Laurie S. Lester, *Charles A. Salter, and *Edward Hirsch. Institute of Chronobiology, Dept. of Psychiatry, New York Hospital-Cornell Medical Center, White Plains, NY and *US Army Natick RD&E Center, Natick, MA.

11:45
112 HORMONAL RHYTHMS IN DEPRESSED PATIENTS. Eric Souêtre[1,2], Edouard Salvati[2], Jean-Luc Belugou[2], Bernard Krebs[2], Guy Darcourt[2]. [1]Pharmacologie clinique, Hopital Necker, 161 rue de Sèvres, 75730 Paris Cedex 15 France. [2]Clinique de psychiatrie et de psychologie médicale, Service du professeur Guy Darcourt, Hôpital pasteur, 06002 Nice Cedex France.

12:00
113 SHORTER SUBJECTIVE SLEEP OF HIGH SCHOOL STUDENTS FROM EARLY COMPARED TO LATE STARTING SCHOOLS. Richard Allen and Jerry Mirabell. The Johns Hopkins University Sleep Disorders Center.

12:15
114 TREATMENT OF PERSISTENT SLEEP-WAKE SCHEDULE DISORDERS WITH METYL-COBALAMIN (VITAMIN B12). T. Ohta, K. Ando, T. Iwata, M. Terashima, Y. Kayukawa, T. Okada, and Y. Kasahara. Dept. of Psychiatry, Nagoya University School of Medicine, Nagoya, Japan.

10:30-12:30 Room B
Slide Session 9
Photic and Non-photic Manipulation of the Clock
Chairperson: Dale Edgar

10:30
115 NON-PHOTIC PHASE RESPONSE CURVES IN WILD TYPE AND *TAU* MUTANT HAMSTERS. N. Mrosovsky, Martin R. Ralph, and Michael Menaker. Depts. of Zoology and Psychology, University of Toronto, Toronto, Ontario, Canada M5S 1A1, and Dept. of Biology, University of Virginia, Charlottesville, VA 22901 USA.

10:45
116 QUANTITATIVE ASSESSMENT OF EXERCISE DEPENDENT ENTRAINMENT IN THE MOUSE. Dale M. Edgar, Connie E. Martin, and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA.

11:00
117 CYCLOHEXIMIDE BLOCK OF LIGHT-INDUCED PHASE SHIFTS IN THE ACTIVITY RHYTHM OF THE HAMSTER. Alfred B. Lord, Joseph S. Takahashi, and Fred W. Turek. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

11:15
118 EFFECTS OF CONTINUOUS ILLUMINATION ON THE SENSITIVITY OF THE HAMSTER CIRCADIAN PACEMAKER TO BRIEF LIGHT PULSES. Dwight E. Nelson and Joseph S. Takahashi. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

11:30
119 RED LIGHT PULSES CAUSE PERIOD AFTER-EFFECTS IN THE CLOCK OF THE UNICELLULAR DINOFLAGELLATE *CONYAULAX*. J. Woodland Hastings and Till Roenneberg. Harvard University Biological Laboratories, Harvard University, 16 Divinity Ave., Cambridge, MA 02138.

11:45
120 MELATONIN GUIDES A SENSITIVE PERIOD IN BIRDS. P. Semm, Th. Schneider, P. Thalau, and W. Wiltschko. Dept. of Zoology, University of Frankfurt, F.R.G.

12:00
121 ACUTE INFLUENCE OF MELATONIN ON THE CIRCADIAN MELATONIN RHYTHM. S.M. Yellon. Division of Perinatal Biology, Depts. Physiology and Pediatrics, Loma Linda University, School of Medicine, Loma Linda, CA 92350.

12:15
122 SPECIFICITY OF CIRCADIAN RHYTHMS IN DIFFERENT INBRED STRAINS OF LABORATORY RATS. F. Wollnik, Dept. of Biology, University of Konstanz, D-7750 Konstanz, F.R.G.

10:30-12:30 Room C
Slide Session 10
Photoperiodic and Seasonal Effects
Chairperson: L. Martinet

10:30
123 PHOTOPERIODIC TIME MEASUREMENT IN GOLDEN HAMSTERS: A TEST OF THE DURATION HYPOTHESIS. M. Watson-Whitmyre, C. Rogers, L. Nicholson, M.D. Rollag[1], and M.H. Stetson. School of Life and Health Sciences, University of Delaware, Newark, DE 19716 and [1]Dept. of Anatomy, USUHS, Bethesda, MD 20814.

10:45
124 OCCLUSION OF THE MELATONIN-FREE INTERVAL BLOCKS THE GONADAL RESPONSE TO PROGRAMMED PHASIC INFUSIONS OF MELATONIN IN THE PINEALECTOMIZED MALE SYRIAN HAMSTER. J. Lindsay, E.S. Maywood, J. Karp, J.B. Powers, J. Herbert, and M.H. Hastings. Dept. of Anatomy, University of Cambridge, Downing St. CB2 3DY, U.K.

11:00
125 PHOTOPERIODIC MODULATION OF SEASONAL OBESITY IN HAMSTERS: ARE CORTISOL AND PROLACTIN PHASE SHIFT INVOLVED? Katarina T. Borer, Pamela Johnson, Mary Beth Brosammer, Uma Swamy, and Melva V. Thompson. Dept. of Kinesiology, University of Michigan, Ann Arbor, MI.

11:15
126 REPRODUCTIVE HISTORY AND THE NEUROENDOCRINE RESPONSE TO DAYLENGTH. C.J.I. Woodfill, N.L. Wayne, and F.J. Karsch. Reproductive Sci. Prog. and Dept. of Physiology, University of Michigan.

11:30

127 THE DEVELOPMENT AND ENDOGENOUS NATURE OF SEASONAL RHYTHMS IN DEER. A.S.I. Loudon and B.R. Brinklow, Institute of Zoology, London NW1 4RY, U.K.

11:45

128 ENDOGENOUS CIRCANNUAL RHYTHMS AND PHOTOREFRACTORINESS OF PROLACTIN SECRETION, TESTIS ACTIVITY AND MOULT IN THE MINK. L. Martinent and R. Monnerie. Laboratoire de Physiologie Sensorielle, INRA, 78350 Jouy en Josas, France.

12:00

129 OPTIMAL LIGHT SENSITIVITY — ITS SIGNIFICANCE FOR MEASUREMENT OF DAYLENGTH. H. Pohl. Max-Planck-Institute für Verhaltensphysiologie, Vogelwarte D-8138 Andechs, F.R.G.

12:15

130 STIMULATION OF REPRODUCTION IN SEASONALLY REPRODUCTIVELY REGRESSED ANIMALS BY DIETARY DIHYDROXYPHENYLALANINE. John M. Wilson and Albert H. Meier, Dept. Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

16:00-18:00 Room A

Workshop 9

Maternal-Fetal Communication of Photoperiodic Information

Teresa Lee (Leader)

Terry Horton, David Weaver, Fred Davis

16:00-18:00 Room B

Workshop 10

Applications of Chronobiology to Cancer Medicine

William J. M. Hrushesky (Leader)

Robert Klevecz, Reinhard Roemeling, Robert Diasio

16:00-18:00 Room C

Workshop 11

Anatomy of Mammalian Circadian Rhythm Regulation

Larry Morin (Leader)

Joke Meijer, Rae Silver, Alan Watts

16:00-18:00 Room 4 & 5

Workshop 12

Modeling of Invertebrate Oscillator Networks

W. Otto Friesen (Leader)

Daniel Hartline, Bard Ermentrout, Ronald Calabrese

18:00-20:00 Room 1-3

Poster Presentations — Group B

Pharmacological Analysis of Rhythms

131 THE EFFECT OF PHARMACOLOGICAL MANIPULATION OF CALCIUM CHANNELS ON *IN VITRO* 2-DEOXYGLUCOSE UPTAKE OF THE SCN. V.H. Cao, J.D. Miller, and T. Kilduff. Depts. of Biological Sciences and Psychiatry, Stanford University, Stanford, CA 94305.

132 REGIONAL VARIATION IN 2-DEOXYGLUCOSE UPTAKE IN THE SCN: EFFECTS OF VIP ANTAGONISM. H.C. Heller, J.D. Miller, V.H. Cao, and T.S. Kilduff. Depts. of Biological Sciences and Psychiatry, Stanford University, Stanford, CA 94305.

133 QUIPAZINE (A SEROTONIN AGONIST) PHASE-SHIFTS THE MAMMALIAN CIRCADIAN CLOCK *IN VITRO*. R.A. Prosser, J.D. Miller, and H.C. Heller. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

134 EFFECTS OF THE NORADRENERGIC NEUROTOXIN DSP-4 ON FREE-RUNNING CIRCADIAN ACTIVITY RHYTHMS. Alan M. Rosenwasser, Dept. of Psychology, University of Maine.

135 CHLORDIAZEPOXIDE-INDUCED PHASE ADVANCES IN SYRIAN HAMSTERS: A BEHAVIORAL AND IN VITRO ELECTROPHYSIOLOGICAL STUDY. S.M. Biello, M.E. Harrington, and R. Mason*. Dept. of Psychology, Smith College, Northampton, MA 01063 USA and Dept. of Physiology, Medical School, Queen's Medical Centre, Nottingham, U.K.

136 CHRONIC ANTIDEPRESSANT DRUG TREATMENT ALTERS THE FLUENCE RESPONSE CURVE FOR PHASE-SHIFTING THE CIRCADIAN PACEMAKER OF SYRIAN HAMSTERS. W.C. Duncan, Jr., P.G. Sokolove, T.A. Wehr. Clinical Psychobiology Branch, NIMH, Bethesda, MD. and the Dept. of Biological Sciences, University of Maryland, Baltimore, MD.

137 CARBACHOL-INDUCED PHASE SHIFTS IN THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY MAY NOT BE DUE TO A DIRECT ACTION ON SCN NEURONS. Beth E.F. Wee, Nick S. Kouchis, and Fred W. Turek. Dept. of Psychology, Tulane University, New Orleans, LA 70118 and Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

138 LIGHT AND HI K+ INDUCED PHASE SHIFTS ARE BLOCKED BY CYCLOHEXIMIDE. B.L. Bogart, S.B.S. Khalsa, G.D. Block. Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

139 RESERPINE WEAKENS THE COUPLING OF TWO CIRCADIAN RHYTHMS IN SCORPIONS. Stephan Michel, Wolfgang Hohmann, and Guenther Fleissner. Zoological Institut, Frankfurt University, Siesmayerstr. 70, D-6000 Frankfurt/Main, F.R.G.

Pineal and Melatonin

140 EVALUATION OF MELATONIN AS A MARKER OF ENDOGENOUS CIRCADIAN PHASE USING A RADIOIMMUNOASSAY (RIA) FOR MELATONIN IN HUMAN PLASMA. T.L. Shanahan and C.A. Czeisler. Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

141 MELATONIN ACTS IN THE MEDIAL BASAL HYPOTHALAMUS TO CONTROL REPRODUCTION IN THE EWE. Benoît Malpaux, Agnès Daveau, Véronique Gayrard, Françoise Maurice, and Jean-Claude Thiery. INRA, Physiologie de la Reproduction, 37380 Nouzilly, France.

142 PINEALECTOMY OF THE PREGNANT EWE DOES NOT ABOLISH THE CIRCADIAN RHYTHM IN FETAL AND MATERNAL PLASMA CONCENTRATIONS OF PROLACTIN. I.C. McMillen, D.W. Walker, I.R. Young, and R. Nowak. Dept. of Physiology, Monash University, Clayton, Victoria, 3168. Australia.

143 RESTRICTED ACCESS TO MOTHER SHIFTED CIRCADIAN RHYTHM OF SEROTONIN N-ACETYLTRANSFERASE (NAT) ACTIVITY IN RAT PUPS. M. Sugishita, Y. Takeuchi, M. Takashima, and K. Takahashi: Div. Mental Disorder Res. National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan (187).

144 MELATONIN INFUSIONS IN WHEEL-RUNNING SIBERIAN HAMSTERS IN CONSTANT LIGHT: EFFECTS ON REPRODUCTIVE STATE AND HYPOTHALAMIC LHRH. Eve S. Hiatt[1], Marie C. Kerbeshian[2], and Janet M. Darrow[2]. Tufts University School of Medicine, Boston, MA[1] and Wellesley College, Wellesley, MA[2].

145 MELATONIN INFUSIONS IN WHEEL-RUNNING SIBERIAN HAMSTERS IN CONSTANT DARKNESS: POSSIBLE ENTRAINMENT OF THE LOCOMOTOR ACTIVITY RHYTHM. Janet M. Darrow and Susan E. Doyle. Dept. of Biological Sciences, Wellesley College, Wellesley, MA 02181.

146 NEUROPEPTIDE-Y REGULATION OF MELATONIN SYNTHESIS IN RAT PINEALOCYTE CULTURES. James Olcese. Institute for Hormone and Fertility Research, Grandweg 64, 2000 Hamburg 54, F.R.G.

147 MELATONIN INHIBITS cAMP PRODUCTION VIA A PERTUSSIS TOXIN-SENSITIVE MECHANISM. L.L. Carlson, D.R. Weaver, and S.M. Reppert. Lab of Developmental Chronobiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

148 THE SITES OF MELATONIN'S ACTION IN THE BRAIN OF THE HOUSE SPARROW, *PASSER DOMESTICUS*. Vincent M. Cassone and David S. Brooks. Dept. of Biology, Texas A&M University, College Station, TX 77843.

149 DAILY AND CIRCADIAN PATTERNS IN 2-[^{125}I]-IODOMELATONIN BINDING IN SPECIFIC SITES OF CHICK BRAIN. David S. Brooks and Vincent M. Cassone. Dept. of Biology, Texas A&M University, College Station, TX 77843.

150 TEMPORAL PROFILE OF SUPEROXIDE DISMUTASE ACTIVITY IN THE PINEAL GLAND. J. Cipolla-Neto, D.S.P. Abdalla, R.P. Markus, and A. Campa. Instituto de Ciencias Biomédicas and Faculdade de Ciencias Farmaceuticas. Universidade de São Paulo, CEP 05508, Brazil.

151 INITIAL CHARACTERIZATION OF A *XENOPUS LAEVIS* RETINAL MELATONIN DEACETYLASE. Michael S. Grace and Joseph C. Besharse. Dept. of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66103, and Dept. of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

152 Listed as 82B.

153 PHOTOTRANSDUCTION IN CULTURED TROUT PINEAL. M. Max, M. Menaker. Biology, University of Virginia, Charlottesville, VA.

Photoreceptors, Pacemakers and Pathways

154 EFFECTS OF CONTINUOUS DARKNESS ON ELECTROETINOGRAPHIC CORRELATES OF PHOTORECEPTOR DISC SHEDDING RABBIT. Mary P. White and Peggy A. Hock. Division of Ophthamology, Veterans Administration Medical Center, Palo Alto, CA 94304.

155 ANALYSIS OF CIRCADIAN PHOTORECEPTORS IN RETINALLY DEGENERATE MICE. R.G. Foster, D. Hudson, W.J. De Grip* and M. Menaker. Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901 USA; *Dept. of Biochemistry, Center for Eye Research, University of Nijmegen, NL-6500 HB Nijmegen, The Netherlands.

156 PATHWAYS CARRYING CIRCADIAN INFORMATION THAT MODULATES ERG AMPLITUDE IN THE RETINA OF THE DIURNAL LIZARD, *ANOLIS CAROLINENSIS*. C. Raul Collazo, Andrew P. Shaw, and Chester J. Karwoski. Vision Research Laboratory, Dept. of Psychology, University of Georgia, Athens, GA

157 CIRCADIAN RHYTHM OF SCN NEURONAL ACTIVITY IN PHOTO-RESPONSIVE AND PHOTO-NONRESPONSIVE DJUNGARIAN HAMSTERS RECORDED *IN VITRO*. Russell R. Margraf and G. Robert Lynch. Dept. of Biology, Wesleyan University, Middletown, CT 06457, USA.

158 RETINAL PROJECTIONS TO HYPOTHALAMIC, THALAMIC AND TELENCEPHALIC AREAS OF THE MINK. J. Peytevin, L. Martinet, J. Servière. Laboratoire de Physiologie sensorielle INRA 78350 Jouy en Josas, France.

159 THE SHEEP SCN: NEUROPEPTIDE/NEUROTRANSMITTER DISTRIBUTION AND RETINOHYPOTHALAMIC INPUT. Q. Gong, R.B. Norgren, Jr., S.M. Moenter, F.J. Karsch, and M.N. Lehman. Dept. Anatomy and Cell Biology, University of Cincinnati, Rep. Science Program, University of Michigan.

160 EFFERENT PROJECTIONS OF THE SUPRACHIASMATIC NUCLEI TO THE FOREBRAIN IN THE SYRIAN HAMSTER *(MESOCRICETUS AURATUS)*. T.G. Youngstrom, M.L. Weiss, and A.A. Nunez. Michigan State University, Neuroscience Program and Dept. of Psychology, E. Lansing, MI 48824.

161 DAILY VARIATION IN MUSCARINIC BINDING SITES IN THE CORTEX AND HYPOTHALAMUS OF GOLDEN HAMSTERS. K.G. Bina[1], M. Wilkinson[2], and B. Rusak[1]. Dept. of [1]Psychology and Dept. of [2]Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.

162 $GABA_A$ AND BENZODIAZEPINE BINDING IN ADULT AND DEVELOPING RAT SUPRACHIASMATIC NUCLEUS. Miqun Li and Jannon L. Fuchs. University of North Texas, Dept. of Biological Sciences, Denton, TX 76203.

163 SEROTONIN RECEPTOR SUBTYPES IN THE SUPRACHIASMATIC NUCLEUS. R.R. Dean, J.D. Miller, and W.C. Dement. Stanford Sleep Disorders Center, 701 Welch Rd., Suite 2226, Palo Alto, CA 94304.

164 REGIONAL DISTRIBUTION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVITY IN THE DJUNGARIAN HAMSTER BRAIN. Marilyn J. Duncan and Willis K. Paull. Dept. of Anatomy and Neurobiology, University of Missouri Medical School, Columbia, MO 65212.

165 LESIONS OF THE SUPRACHIASMATIC NUCLEUS (SCN) THAT DISRUPT MELATONIN SECRETION MAY NOT BLOCK RESPONSES TO PHOTOPERIOD SHIFTS. G.L. Jackson, C. Kao, H.T. Jansen. Dept. of Veterinary Biosciences, University of Illinois.

166 PERSISTENCE OF ACTIVITY PATTERNS FOLLOWING SCN LESIONS. P.J. Sollars and G.E. Pickard. Dept. of Anatomy, West Virginia University, Morgantown, WV.

167 CAN TIME OF IMPLANTATION AFFECT OUTCOME OF SUPRACHIASMATIC NUCLEUS TRANSPLANT? M.A. Vogelbaum and M. Menaker. Division of Biomedical Engineering and Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

168 WHICH NEUROPEPTIDES/NEUROTRANSMITTERS ARE ASSOCIATED WITH SCN GRAFTS THAT RESTORE RHYTHMICITY TO HAMSTERS? Stephen McKeehan, Eric L. Bittman, and Michael N. Lehman. Dept. Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH and Dept. Zoology, University of Massachusetts, Amherst, MA.

169 RETINOHYPOTHALAMIC TRACT (RHT) AND SYNAPTOGENESIS IN THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN). Joan C. Speh and Robert Y. Moore. Depts. of Neurology and Neurobiology, State University of New York at Stony Brook, Stony Brook, NY 11794.

170 FUNCTIONAL AND TEMPORAL NEUROANATOMICAL MAPPING OF INSECT CLOCK. M. Lavialle and B. Dumortier. INRA — Laboratoire de Physiologie Sensorielle — 78350 Jouy en Josas, France.

Cellular and Molecular Basis of Rhythmicity

171 A REEXAMINATION OF THE ROLE OF THE NUCLEUS IN GENERATING THE CIRCADIAN RHYTHM IN *ACETABULARIA*. John C. Woolum. Dept. of Physics and Astronomy, California State University, Los Angeles, CA.

172 REGULATION OF GENES UNDER CONTROL OF THE CIRCADIAN CLOCK. Kristin A. Lindgren, Ann Lichens-Park, Jennifer J. Loros, and Jay C. Dunlap. Dept. of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

173 PORTIONS OF THE *NEUROSPORA frq* PROTEIN SHOW SIMILARITY WITH NUCLEAR LOCALIZATION SIGNALS. M.T. Lewis and J.F. Feldman. University of California, Santa Cruz, Santa Cruz, CA 95064.

174 MOLECULAR ANALYSIS OF THE *FREQUENCY AND PERIOD-4* LOCI OF NEUROSPORA. Keith A. Johnson, Q. Liu, and Jay C. Dunlap. Dept. of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

175 CIRCADIAN RHYTHMS IN *NEUROSPORA*: PHASE-RESPONSE CURVES AND OSCILLATOR AMPLITUDE IN CLOCK MUTANTS. Stuart Brody, Dept. of Biology, University of California, San Diego, La Jolla, CA; Patricia Lakin-Thomas. Dept. of Botany, Cambridge University, Cambridge, UK; Gary Coté. Dept. of Molecular and Cell Biology. University of Connecticut, Storrs, CT.

176 IMMUNOHISTOLOGICAL STAINING OF A *PER*-LIKE PROTEIN IN THE BEETLE, *PACHYMORPHA SEXGUTTATA*. Brigitte Frisch[1], Jeffrey C. Hall[1], Michael Rosbash[1,2], Gerta Fleissner[3], and Günther Fleissner[3]. [1]Dept. of Biology, and [2]Howard Hughes Medical Institute, Brandeis University, Waltham, MA 02254-9110, USA. [3]Zoologisches Institut, Universität Frankfurt, Siesmayerstr. 70, 6000 Frankfurt, F.R.G.

177 "PER"-REACTIVE NEURONS IN THE BRAIN OF THE SCORPION *ANDROCTONUS AUSTRALIS*. G. Fleissner, B. Brandes-Frisch*, J. Hall*. Zoologisches Institut der Universität Frankfurt/Main, Siesmayerstr. 70, D-6000 Frankfurt/Main, F.R.G. and (*)Biology Dept., Brandeis University, Waltham, MA USA.

178 REVERSIBLE TRANSCRIPTION INHIBITOR ALTERS PERIOD AND PHASE OF A CIRCADIAN RHYTHM AND BLOCKS SOME EFFECTS OF LIGHT ON PROTEINS. S. Ramasubban and A. Eskin. Dept. of Biochemistry and Biophysics Science, University of Houston, Houston, TX 77204.

179 AMINO ACID SEQUENCE AND FUNCTION OF A PUTATIVE CIRCADIAN OSCILLATOR PROTEIN. U. Raju, M. Nunez-Regueiro, *R. Cook, and A. Eskin. Dept. of Biochemistry and Biophysics Science, University of Houston, *Baylor College of Medicine, Houston, TX 77204.

180 A POSSIBLE MECHANISM OF PERIOD SHORTENING BY CHLORIDE CONDUCTANCE INHIBITION IN THE *BULLA* OCULAR CIRCADIAN PACEMAKER. S.B.S. Khalsa, S. Michel, and G.D. Block. Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

181 CIRCADIAN VARIATIONS OF MESSENGER RIBONUCLEIC ACID FOR PEPTIDE SOMATOSTATIN IN THE SUPRACHIASMATIC NUCLEUS. Shin-Ichi T. Inouye, Hiroaki Nagasaki, Junichi Takeuchi, and Ako Tokumasu. Lab. Neurophysiology, Mitsubishi Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, Japan.

182 LIGHT-INDUCED C-FOS EXPRESSION IN THE SCN IS PHASE DEPENDENT. M.A. Rea, Neuroscience Laboratory, Aerospace Research Branch (VNB), USAF School of Aerospace Medicine, Brooks AFB, TX 78235.

183 REGULATION OF THE LENGTH OF THE CELL CYCLE AND ITS PHASES BY THE LIGHT/DARK SCHEDULE. M.L. Wright, S. Jorey, L. Blanchard, L. Garatti, and S.M. Mayrand. College of Our Lady of the Elms, Chicopee, MA.

1 LITHIUM ALTERS THE CIRCADIAN RHYTHM OF DISK-SHEDDING AND ENHANCES LIGHT EFFECTS IN RAT RETINAL PHOTORECEPTORS. Ch. Remé, U.Braschler, and K. Munz, University Eye Clinic, Zuerich, Switzerland

Lithium phase delays and dampens circadian rhythms at different levels of biological function and interacts with inositollipid derived second messengers in the central nervous system. We have tested the effect of lithium application on the circadian rhythm of disk-shedding and light elicited disk-shedding responses as well as the susceptibility to light induced photoreceptor lesions and the light-dependence of lithium uptake in the retina. In male albino rats of 250 gr, lithium treatment (2.6g/kg chow; serum-lithium levels 0.8-1.1 mmol/l) for 3 weeks significantly dampens the disk-shedding rhythm in constant darkness (DD) but not in a light-dark cycle (LD 12:12, L=5-10 lux) and enhances light-elicited disk-shedding responses at different times of the LD cycle. Further, the susceptibility to acute photoreceptor lesions induced by LL of different illuminance levels or light pulses (100 -1000 lux, 30 min.) is distinctly increased. Lithium is selectively accumulated in the retina compared to other ocular tissues and serum. Uptake of lithium in the retina as compared to other ocular tissues and serum is dependent on lighting conditions with high levels in DD (72 h) and minor amounts in LL (500 lux, 72 h). Preloaded lithium levels, however, do not change with varying light exposure conditions.Thus, photoreceptor structure, biochemistry (Pfeilschifter et al., 1988) and circadian rhythms are altered by chronic lithium application suggesting that the visual input signal to the central circadian pacemaker in the suprachiasmatic nuclei may be modified.

2 COMPARATIVE STUDY OF ULTRADIAN AND CIRCADIAN BIORHYTHMS OF NORMAL RATS AND RATS WITH EXPERIMENTAL NEUROSIS

J.Drescher, K.Wicht, G.Haupt, R.Weigel, A.Tsikadse, H.Weissleder
Institut of Pathophysiology / Charite, Humboldt-University Berlin
Ziegelstrasse 5-9 , Berlin 1 0 4 0 , GDR

Using a complex measuring system for the long term registration of :
- rhythms of body and brain temperature,
-dynamics of CO/2-O/2 in the respiration air,
- spontaneous locomotion activity and
- the EEG and EMG pattern,
we analyzed the sleep-wakefulness-cycles in connection with the biorhythms of the vegetative and behavioral parameters under L:D = 12:12 h illumination conditions and constant temperature 24°C. We compared the results of normal rats (N=5) and rats with experimental information neurosis (N=5 / Model of the Pathology of higher nervous functions / Chananashvili M.M. - Inst. of Physiology I.S.Beritashvili Tbilisi). We demonstrated the alterations of the interconnections between circadian and ultradian biorhythmic profiles by using the Auto- and Crosscorreltion functions, Fourier-Analysis, Periodogramanalysis and Cosinorrhythmometry.

We found that the coupling relationships of circadian and ultradian biorhythms are disturbed in neurotic rats. There are tendencies to more shorter rhythms.

3 A GENETIC ANIMAL MODEL FOR STUDIES OF DEPRESSION AND DISTURBED CIRCADIAN RHYTHMS Gail Orpen and Meir Steiner Neurobiology Laboratory, St. Joseph's Hospital Research Institute, and Department of Psychiatry, McMaster University, Hamilton, Ontario, Canada.

The objective of our research is to develop a pigmented strain of rat which has a genetically-determined cholinergic supersensitivity, to be used as an animal model in studies of functions such as light sensitivity and circadian regulation which are disturbed in affective disorders. For this purpose, we have crossbred the Wistar-Albino-Glaxo (WAG) rat, which is supersensitive to the effects of muscarinic agonists, with the pigmented Dark Agouti (DA) rat.

The WAGxDA F1 offspring are more sensitive than DAs to the temperature-depressing effects of cholinergic agonists, as demonstrated by a greater drop in core body temperature 30 minutes after being given oxotremorine (.25 mg/kg, i.p.) in the presence of peripheral receptor blockade by methyl atropine. Body temperature dropped significantly more in WAGxDA than in DA rats (Males: 2.25 C$\pm$0.41 vs. 1.84$\pm$0.56, df=46, $p<0.01$; Females: 2.85$\pm$0.49 vs.2.03$\pm$0.48, df=27, $p<0.01$). Also females showed a greater temperature drop than males (df=53, $p<0.01$).

Data on muscarinic receptor binding in different brain areas, as well as post-receptor functioning (GTP binding) and behavior in WAGxDA vs DA females will be presented.

(Supported by the St. Joseph's Hospital Foundation.)

4 EFFECT OF IMIPRAMINE, AMITRIPTYLINE AND DIAZEPAM ON CIRCADIAN RHYTHMS IN THE FIELD MOUSE Mus booduga

P SUBRAMANIAN, Department of Animal Behaviour, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021 INDIA

Experiments on the influence of imipramine and amitriptyline offered through drinking water, on the period length (τ) of the rhythm in the wheel running activity of field mouse Mus booduga were performed under continuous darkness (DD). Ingestion of imipramine to mice under free running conditions modulates the period length (τ) of the circadian activity rhythm. Furthermore, the activity time (α) is significantly reduced ($p < 0.05$) during the days of imipramine as well as amitriptyline treatment. It is hypothesized that fast circadian oscillator or pacemaker might contribute to affective illnesses, particularly manic depressive disease. Modulation of oscillators' frequency by these antidepressants supports the notion that their therapeutic action is to delay the overtly fast circadian rhythms.

A Phase Response Curve (PRC) was constructed by giving two hour tonic pulses of diazepam in drinking water, at various phases (circadian times), to the free running animals. Phase advances are observed during early subjective day. Phase delays are observed during the remaining phases. The diazepam PRC is not similar to the quality of light pulse PRC obtained for Mus booduga (unpublished). The phase shifting action of diazepam (a benzodiazepine) may be explained by its agonistic (potentiating) action of the neurotransmitter, gamma aminobutyric acid (GABA).

5 ALTERED WAVEFORM OF PLASMA NOCTURNAL MELATONIN SECRETION IN PREMENSTRUAL DEPRESSION

BL Parry, SL Berga, GA Laughlin, MR Klauber, SSC Yen, DF Kripke, JC Gillin

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Premenstrual Syndrome (PMS) and affective disorders may be related illnesses. Since chronobiological abnormalities have been implicated in the pathogenesis of affective illness, we wished to determine, by using melatonin as a marker for circadian phase, whether similar chronobiological abnormalities occurred in PMS.

The nocturnal secretion of plasma melatonin was determined under dim to dark conditions in 8 patients with prospectively confirmed PMS and in 8 age-matched normal control (NC) subjects. Plasma samples for melatonin were collected every 30 minutes from 1800-0900 hours (h) during the early follicular (EF), late follicular (LF), mid luteal (ML) and late luteal (LL) phases of the menstrual cycle.

Compared to NC, PMS patients had an earlier (phase-advanced) offset of melatonin secretion which contributed to a shorter secretion duration and a decreased area under the curve (AUC). No statistically significant differences were found between PMS and NC women for melatonin onset, or peak concentration, or for estradiol (E_2) or progesterone (P) levels.

The data demonstrate that women with PMS have chronobiological abnormalities of melatonin secretion. The fact that these patients respond to treatments such as sleep deprivation and phototherapy which affect circadian physiology suggests that circadian abnormalities may contribute to the pathogenesis of PMS.

6 EFFECTS OF WARM AND COOL AMBIENT TEMPERATURES ON DEPRESSED PATIENTS' BODY TEMPERATURE, HORMONES AND MOOD DURING SLEEP DEPRIVATION IN A CONSTANT ROUTINE

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Physiological effects of sleep-onset resemble physiological effects of heat-exposure. These similarities are probably not coincidental; they may arise from fundamental links between sleep physiology and thermoregulatory physiology. In fact, sleep-onset and heat-exposure cause essentially equivalent perturbations of hypothalamic thermoregulatory control mechanisms, since lowering of T_{set} after sleep-onset generates the same error signal as raising of T_{hy} during heat-exposure. In patients with affective disorders, extensive experimental work has shown that sleep can induce depression and that sleep-deprivation can improve depression and induce mania. We are investigating the possible role of thermoregulatory mechanisms in these responses to sleep and sleep-deprivation. In an ongoing experiment, we sleep-deprived twelve depressed patients for forty hours on two separate occasions, once at T_a = 33° C, and once at T_a = 18° C (RH held constant at 60%). During a baseline 24-hour period, and during the last 24 hours of the two sleep-deprivation periods, using a constant-routine-method, we monitored rectal temperature, motor activity, EEG, and clinical state, and we obtained hourly blood samples for measurement of prolactin (PRL), thyrotropin (TSH), Free T_3 (FT_3), cortisol, and melatonin. Rectal temperature was consistently 0.5° C higher in the warm condition compared with the cool condition. Neuroendocrine and clinical effects of sleep deprivation (inhibition of PRL, stimulation of TSH and FT_3, and improvement of depression) were attenuated in the warm T_a compared with the cool T_a. These results can be interpreted as supporting the hypothesis that the heat-like properties of sleep are responsible for its depressant effect in patients with affective disorders.

7 **24-HOUR CORTISOL SECRETORY PATTERNS IN DEPRESSED ADOLESCENTS**

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There has been considerable interest in circadian regulation with respect to major depressive disorder (MDD). The accelerated onset of REM sleep is a robust psychobiologic marker of depression in adult subjects, and additional evidence has indicated a phase advance of cortisol and temperature rhythms. Our work has focussed on sleep and neuroendocrine measures in the child and adolescent age groups with MDD compared to age matched normal controls.

The sample in this study consists of 27 adolescents meeting DSM-III diagnostic criteria for major depressive disorder and 30 age matched carefully screened normal, healthy control adolescents. Adolescence was defined as at least Tanner Stage III of sexual development, but under 18 years of age. All subjects came into the Child & Adolescent Sleep & Neuroendocrine Lab for a three day psychobiologic protocol with three consecutive nights of polysomnographic sleep recordings. On the third day, an intra-venous catheter was inserted and plasma cortisol levels were measured every 20 minutes for the next 24 hours.

There were no between group differences in 24-hour mean cortisol, peak cortisol level, or time of cortisol rise at night. A plot of 24 hour values showed a remarkably similar pattern of cortisol between the two groups, except for the time between 8:00 p.m. and 11:00 p.m. During this interval, the normal group showed very low levels of cortisol (below 2mcg/dl) while the depressed group averaged 4 mcg/dl. The absence of a quiescent period in the depressed group also resulted in higher cortisol values within 180 minutes of sleep onset compared to the normal controls.

We conclude that there is no evidence for circadian differences in cortisol secretion in this adolescent depressed group, only an increase in hypotholamic-pituitary-adrenal axis activity at the normal quiescent time from 8:00 to 11:00 p.m.

8 **SEROTONERGIC EFFECTS ON CORTISOL SECRETORY PATTERNS IN DEPRESSED CHILDREN**

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The role of circadian dysregulation in major depressive disorder (MDD) has been the source of considerable research and speculation. The accelerated onset of REM sleep is one of the robust psychobiologic markers of depression in adult subjects, with additional evidence suggesting a phase advance of cortisol and temperature rhythms. Our work has focussed on sleep and neuroendocrine measures in the child and adolescent age groups with MDD compared to age matched normal controls. This sample consisted of prepubertal children who met DSM-III criteria for major depressive disorder and a group of carefully screened healthy normal control children, enrolled in a psychobiologic study of sleep and neuroendocrine function. All subjects were between 6 and 12 years of age and Tanner Stage I or II of sexual development. Following an adaptation night in the sleep unit, baseline measures of sleep and 20 minute samples of plasma cortisol were obtained. Later in the protocol, the same sleep and plasma hormone measures were repeated in conjunction with an intervenous infusion of L-5-Hydroxtryptophan (5HTP), a serotonergic precursor.

During the baseline night, the latency from sleep onset to rise in cortisol was significantly delayed in the depressed children compared to age matched normal controls. There were no differences in time of sleep onset or REM onset. On the night of the L-5HTP infusion, these group differences in cortisol latency disappeared.

In contrast to adult studies which suggest a phase advance of cortisol rhythm in association with episodes of depression, these studies suggest a phase delay of cortisol in association with depression in children. In addition, correction of this delay in cortisol rise by a serotonergic precursor lends further evidence for decreased central serotonergic activity in association with major depressive disorder.

9 A MATHEMATICAL MODEL FOR THE INSULIN-GLUCOSE FEEDBACK MECHANISM ACCOUNTS FOR THE EXISTENCE OF ULTRADIAN OSCILLATIONS OF HUMAN INSULIN SECRETION. J. Sturis, K.S. Polonsky, E. Mosekilde, E. Van Cauter. Technical University of Denmark, DK-2800 Lyngby, Denmark- and- Dept of Medicine, University of Chicago, IL 60637, USA.

In man, glucose levels and insulin secretion exhibit ultradian oscillations with a period of 100-150 min. The mechanisms generating these oscillations are not known. To determine whether the feedback structure of the insulin-glucose system could be causing this oscillatory behavior, we developed a parsimonious mathematical model describing the effects of insulin on glucose production and glucose utilization and the stimulatory effect of glucose on insulin secretion. The model involves three main variables: the amount of glucose in its distribution volume, the amount of insulin in plasma and the amount of insulin in the interstitial fluid. Three additional variables are used to model the delay between the appearance of insulin in plasma and glucose production. The relations between variables and the values for the parameters of the model were estimated from previously published human in vivo data. Two features of the model were found to be essential for the existence of oscillations: 1. the delay of 30-45 min between the appearance of insulin in plasma and its effect on glucose production; 2. the fact that glucose utilization depends on insulin levels in a compartment remote from plasma. The results of extensive model simulations mimicked all experimental findings so far obtained for these ultradian oscillations including: 1. damped oscillations after oral glucose ingestion, 2. self-sustained oscillations during constant glucose infusion, 3. increased amplitude, but not frequency, when the rate of glucose infusion is increased, 4. 10-20 min advance of the glucose oscillation as compared to the insulin oscillation. The model also predicts limits of entrainment of the endogenous oscillations by oscillatory infusion of exogenous glucose which have been confirmed by a series of recent experiments. In conclusion, the existence of ultradian oscillations of human insulin secretion and glucose levels and their functional properties may be entirely accounted for by the major dynamic characteristics of the insulin-glucose feedback loop.

10 ENTRAINMENT OF ULTRADIAN OSCILLATIONS OF HUMAN GLUCOSE AND INSULIN BY OSCILLATORY GLUCOSE INFUSIONS. K.S. Polonsky, J. Sturis, J.D. Blackman, E. Van Cauter. Dept of Medicine, University of Chicago, IL 60637, USA-and- Technical University of Denmark, DK-2800 Lyngby, Denmark.

In man, the existence of ultradian oscillations (period: 100-150 min) of glucose levels and insulin secretion has been recently recognized. The mechanisms causing these oscillations have yet to be elucidated. In particular, it is not known whether glucose plays an active role in generating the oscillations, or if an independent intra-pancreatic pacemaker generates oscillations of insulin secretion which force glucose to oscillate passively and in synchrony with insulin. To distinguish between these alternative mechanisms, six normal subjects were studied each on three different occasions using different patterns of glucose infusion. First, each subject received a constant infusion for 28 hrs at a rate of 6 mg/kg/min. This study served to determine the endogenous period of oscillation of each subject individually. On two subsequent occasions, the subjects received an oscillatory glucose infusion for 28 hrs with the same mean rate, an amplitude of ± 33% of the mean, an approximately sinusoidal waveshape, and a period either 20% longer or 20% shorter than the endogenous period. Based on a mathematical model of the insulin-glucose feedback loop, these periods had been estimated to be within the range of entrainment. In each study, blood was sampled every 10 min during the last 24 hrs of the infusion and glucose, insulin and C-peptide levels were measured on each sample. In all subjects, oscillations of glucose, insulin and C-peptide entrained to the exogenous period. No significant glucose-independent oscillatory activity of C-peptide and insulin could be identified. Additional experiments were performed to determine the response to exogenous periods outside the range of entrainment. When the exogenous period was twice as long as the endogenous period, exactly two endogenous oscillations occurred for each exogenous oscillation. These results strongly indicate that glucose plays an active role in generating the ultradian oscillations of insulin secretion and that it is not necessary to postulate the existence of an inherent intra-pancreatic pacemaker.

11 DIETARY FIBER DOES NOT MODIFY PLASMA GLUCOSE AND INSULIN PROFILES IN NORMAL AND DIABETIC SUBJECTS UNDER CONTINUOUS ENTERAL NUTRITION.

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Peripheral plasma concentrations of glucose and insulin were measured at 10-min intervals in 6 patients with noninsulin-dependent diabetes mellitus and 6 matched controls during continuous enteral nutrition for a 12-h period. Each subject was studied twice with a standard solution (86 Kcal.h^{-1} ; 50% carbohydrate ; 35% fat ; 15% protein) containing either 6.3 g or 17 g vegetable fibers per 1000 kcal.

In the control subjects, mean plasma glucose and insulin concentrations rose sharply and then attained a steady state ; in the diabetic patients, mean insulin concentrations were lower (132.0 + 12.8 vs 188.0 + 29.4 pmol/L) contrasting with higher glucose levels (15.5 + 2.0 vs 6.0 + 0.2 mmol/L). The mean insulin profile was characterized by a slow continuous ascending trend throughout the experimental day. These profiles were not significantly affected by addition of fiber.

Analysis of the individual 12 h profiles revealed that plasma glucose levels had a similar oscillatory pattern in the diabetic subjects and the controls (7.2 + 0.5 vs 9.0 + 0.6 pulses produced in each 12 h period). In contrast, the number of insulin pulses was significantly lower in the diabetic patients (3.5 + 0.4 vs 7.5 + 0.4 pulses produced in each 12 h period) ; they had a smaller amplitude (55.2 + 7.2 vs 101.2 + 26.5 pmol/L) and were poorly associated with the glucose oscillations (37% vs 77%). Neither glucose nor insulin pulse characteristics were modified by the addition of fibers.

This work was supported by Wander A.G.

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OBESITY, HYPERINSULINEMIA AND INSULIN RESISTANCE ARE DIMINISHED BY RESETTING CIRCADIAN NEUROENDOCRINE RHYTHMS. A. H. Meier and A. H. Cincotta, Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge La. 70803

A causal role for altered circadian rhythms in obesity is indicated by differences in the phase relationships of several hormone rhythms in lean and fat animals. Furthermore, daily injections of corticosteroid hormone and prolactin stimulate increases or decreases in body fat stores as a function of the times of their injections. In female Syrian hamsters, prolactin injections given 12 hours after daily corticosteroid injections support fattening whereas prolactin injections at the same time as corticosteroid injections (0-hour relation) promote losses in body fat. In the male Spraque-Dawley rat, a 0-hour relation supports fattening and a 4-hour relation of hormones (prolactin given 4 hours after corticosteroid) reduces body fat. Dramatic changes in fat stores caused by such timed daily injections for 10 days persist for at least 3-4 months following treatment in both rodent species suggesting that the injections reset circadian neuroendocrine oscillations regulating lipid metabolism. The relations of hormone injections that establish leanness also produce pronounced and long lasting reductions of plasma insulin concentrations and insulin resistance in both rodents. Because obesity, hyperinsulinemia and high insulin resistance are hallmarks of type II (maturity onset) diabetes, our findings further suggest that type II diabetes is caused by altered circadian rhythms and that a resetting of their phase relationships may lead to a cure for this intractable disease.

13 **RESTRICTED FEEDING: MASKING OR/AND ENTRAINMENT?**

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D - 7 9 0 0 U l m / FRG

Unequivocally RF masks circadian rhythms. In the rat, entrainment is limited only to a component of total locomotor activity: this component anticipates RF while the free-running rhythm appears to remain almost unaffected.
There are good reasons to investigate masking or/and entrainment of rabbits:
1. as a strictly herbivorous animal the rabbit has a less discontinuous food-intake pattern than omnivores or carnivores. In addition it has a sophisticated system of retaining and, in a precise circadian rhythm, recycling highly digestible particles of caecal contents. The rabbit thus almost continuously does ingest something. An exogenous schedule of discontinuous food intake therefore might act differently in the rabbit than in omnivores or carnivores.
2. during the first weeks of life rabbit-pups in fact are entrained by 'restricted feeding': the mother contacts the litter only once per day for 3-5 minutes and the pups are in phase with her presence.

In several experiments adult male rabbits in fact were entrained with a four hours food access schedule. The rhythms of locomotor activity, access to the water bottle, urine excretion and hard faeces excretion had coherent phases even during entrainment with RF. The time of entrainment of the free-running circadian rhythm with RF did depend from the phase of RF relative to the phase of the rhythm.
In addition, RF exerted a strong masking-effect, which did camouflage much but not all of the circadian pocesses.

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DAILY RHYTHMS OF CARBOHYDRATE- AND PROTEIN-RICH FOOD CHOICE UNDER DIFFERENT PHOTOPERIODS.

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Ten adult male Wistar rats (*ca.* 500g) were kept individually on the following successive photoperiods (PP): LD 12:12 (5 weeks); skeleton photoperiod (SPP) 12:12 (1 week); LD 8:16 (5 weeks); LD 16:8 (5 weeks). Light intensity was 30 lux at the top of the cages. Two diets were available (monitored in 6' bins via a computerised hopper system): carbohydrate-rich (**CHO**; 62% carbohydrates) and protein-rich (**PROT**; 65% caseine), with water ad lib. Total food intake under different PPs showed no significant changes. The average weight gain was similar in all PPs (0.8±.4 [*sd*] g/day) except winter, where weight was lost (-0.1 ±.4 g/day). For analysis of daily rhythms, only data from the last week of each PP were used.

A comparison of LD 12:12 vs. SPP indicated that light per se had little masking effect. In LD 12:12, CHO intake gradually increased during L to a maximum at the beginning of D; PROT intake remained low during L and rose to a maximum towards the end of D (as has been shown previously). With D of 12h or 16h, both CHO and PROT decreased during the last 2h period before lights on. Under a summer PP, however, nocturnal food intake was compressed, with high values throughout the short dark phase. In the long light phase the gradual increase of CHO intake persisted, whereas PROT intake remained low.

This self-selection study in the behaviourally non-seasonal rat revealed: 1. Growth rate decreases in a winter PP despite food intake comparable to other seasons, indicating that metabolic processes may be altered by different lighting schedules. 2. PRO is always low during the light phase, whereas CHO-intake increases throughout. Thus during its inactive phase, the rat preferentially chooses CHO for direct energy needs.

15 Disruption of circadian rhythmicity in experimental hepatic encephalopathy. PC Zee, R Mehta, AT Blei and FW Turek. Depts of Neurology, Medicine and Neurobiology & Physiology, Chicago and Evanston, IL.

Disruption of circadian rhythmicity has been associated with few human diseases. Chronic liver disease might represent such a condition since during the early stages of hepatic encephalopathy there are prominent disturbances of the sleep/wake cycle. To investigate this possibility, the circadian rhythms of locomotor activity and pineal melatonin content were studied in rats with portacaval anastomosis (PCA), a model for hepatic encephalopathy. In the first study, locomotor activity was recorded for 3 weeks from 18 rats exposed to a light/dark (LD) cycle of 12:12 before animals received either a PCA or sham operation. Post- operatively, animals were kept under similar LD conditions for another 3 weeks before being transferred to continuous light (LL). In a second study, 12 PCA and 12 sham-operated rats were exposed to LD 12:12 for 3 weeks. Pineal glands were removed during the middle of the subjective day or night during the first day of constant darkness.

All sham-operated animals entrained normally to LD and exhibited free-running periods. In contrast, the circadian rhythm of locomotor activity in all PCA rats was clearly disrupted under both entrained and free-running conditions. During exposure to LD, there was a decrease in the amplitude of the diurnal activity rhythm in 4/8 animals, while the remainder showed no significant entrainment. During LL, a circadian rhythm (with decreased amplitude) was detected by chi-square periodogram analysis in 5/8 animals, while no rhythm was apparent in the remaining 3 animals. Normal diurnal variations of pineal melatonin levels were seen in sham-operated animals. However, this normal diurnal variation of pineal melatonin was disrupted in PCA animals. PCA induces marked disturbances of both a behavioral and endocrine circadian rhythm, known to be regulated by a common pacemaker. This suggests that the circadian disturbance associated with PCA originates within the SCN or its afferent/efferent pathways.

16 CIRCADIAN BODY TEMPERATURE (T_b) RHYTHMS PERSIST THROUGHOUT HIBERNATION IN GOLDEN MANTLED GROUND SQUIRRELS. D. A. Grahn, J. D. Miller, C. M. Radeke, V. Houng, and H. C. Heller. Dept. of Biological Sciences, Stanford University, Stanford, Ca. 94305.

To determine whether a circadian T_b rhythm is present throughout hibernation, 22 golden mantled ground squirrels were implanted with temperature sensitive transmitters and their T_b's were monitored from August 1988 through January 1989. The animals were maintained at 10-11°C under constant dim light. Prior to hibernation each animal established an independent free running T_b rhythm with an amplitude of 2-3 °C (ranging from 35-39°C) and a tau between 23 and 25 hours. Two to 5 days before the first hibernation bout, each animal's circadian T_b rhythm was disrupted. Upon initiation of the first hibernation bout, a daily T_b rhythm was re-established. During a hibernation bout (T_b 12 to 14 °C) there was a clear circadian oscillation of T_b with an amplitude of less than 1°C. There was greater range in taus observed during hibernation than during euthermia.

Arousals from hibernation showed a free running rhythm. The times between arousals were multiples of the circadian tau seen during deep hibernation. The euthermic period lasted for approximately 24 hours before the animal entered another hibernation bout. The initiation of the arousal always occurred during the rising phase of the T_b cycle. From these observations we conclude that the circadian system, as manifested by changes in T_b, is maintained during hibernation and is temperature compensated. There is, however, a discontinuity in tau between the euthermic and hibernation seasons. (This research was supported by a grant from the Upjohn Company.)

17 A COMPARISON OF THE PHOTIC SENSITIVITY FOR THE PHASE-SHIFTING AND ACUTE EFFECTS OF LIGHT ON THE OSCILLATION OF MELATONIN RELEASE FROM CHICK PINEAL CELLS. L.M. Robertson and J.S. Takahashi, Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Chick pineal cells exhibit a circadian oscillation of melatonin release. The cells are photoreceptive and light has two major effects: 1) acute inhibition of melatonin release and 2) entrainment of the circadian oscillator. Previous studies have indicated that some of the cellular processes involved in retinal phototransduction are involved in the acute but not the phase-shifting effect of light. In order to characterize this further we explored the effects of different light intensities on both the acute and phase-shifting response. We developed a flow-through cell culture system in which cells were grown in monolayer cultures on multi-well cell culture plates. Medium was pumped through each well via teflon tubing set in a silastic stopper. Two-hr fractions were collected and analyzed for melatonin content using radioimmunoassay. A fiberoptic system underneath the plates provided the photic stimulus. BG-40 filters were used to ensure a consistent broad spectrum of light and neutral density filters were used to adjust the intensity. Cells were exposed to a range of light intensities for 6 hours during the first 24 hr in constant darkness. Both the acute and phase-shifting responses increased in magnitude with an increase in light intensity. The half-maximal intensity for the acute response was approximately 1×10^{6} watts/cm^2 regardless of the circadian time of the light pulse. The intensity relationship of the phase-shifting response to light pulses presented during the early subjective night was similar to that observed with the acute response. However, during the late subjective night, the phase-shifting response appeared to be less sensitive to light than the acute response. These results suggest that different mechanisms may underlie the acute and phase-shifting effects of light on the circadian oscillation of melatonin release from chick pineal cells.

18 SPECTRAL SENSITIVITY OF THE CIRCADIAN CLOCK'S RESPONSE TO LIGHT IN DJUNGARIAN HAMSTERS. Martha M. Hotz, Jill J. Milette, Joseph S. Takahashi, and Fred W. Turek, Dept. Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

The photoreceptors mediating the effects of light on the circadian clock and the reproductive system are located in the eyes of mammals, however, the specific photoreceptor(s) and visual pigment(s) involved have not been identified. In order to define the characteristics of light needed to stimulate the clock's photoreceptors, we determined the spectral sensitivity, or wavelength dependence, for light-induced phase shifts of the locomotor activity rhythm of the Djungarian hamster (Phodopus sungorus). Six week-old male hamsters were singly housed in cages equipped with running wheels and wheel-running was recorded from animals in constant dark for 10 days. Animals were then exposed to a single 15 minute monochromatic light pulse at CT 14 (two hours after activity onset) and then allowed to free-run in constant dark for an additional 10 days. The magnitudes of phase shifts were determined for at least 10 animals per intensity and at least 4 intensities of each wavelength were used. Intensity-response curves were determined for 9 wavelengths of light. The spectral sensitivity curve for the phase-shifting response to light indicates a peak near 475 nm. This is in contrast with published data on phase shifting responses to light in the golden hamster indicating a photoreceptor with a maximum sensitivity near 500 nm (Takahashi et al., 1984, Nature 308: 186.). However, a 475 nm peak is consistant with our previous data on the spectral sensitivity of the reproductive responses to light in Djungarian hamsters (Milette et al., 1987, Biol Reprod 36 suppl 1:110), indicating that a common set of photoreceptors is likely to mediate both responses. These data appear consistent with a photoreceptor type with a peak sensitivity in the blue range, such as a blue cone.

19 **CIRCADIAN ADJUSTMENT TO A SEASONALLY-MODULATED NATURALISTIC LIGHTING ENVIRONMENT.** *Michael Terman, Jiuan Su Terman, & Stephen Fairhurst.* Columbia University Dept. of Psychiatry and New York State Psychiatric Institute, Box 50, 722 W. 168th St., New York, NY 10032.

Rats were individually maintained in an environment that permitted self-exposure to computer-simulated naturalistic lighting cycles with dawn and dusk twilight transitions precisely mimicking summer and winter solstices, and the equinoxes, at 45° N latitude. Two identical chambers *(above, below)*, both of which provided free access to food and water, were connected by a light-locked conduit. A fluorescent light source with rotating vane-attenuation system continuously illuminated the chamber *above* within a range spanning starlight (ca 10^{-4} lux) to daylight (ca 10^{3} lux). The rat was free to shuttle between the chambers at all times. Entrainment was first established under a conventional LD cycle in a separate standard Skinner box. The rat was then transferred to the illuminator system and maintained under a constant seasonal condition for several weeks. Afterwards, the rat was returned to the Skinner box for determination of its free-running period (tau_{DD}).

The animals' patterns of self-exposure to the light followed reliable daily patterns that varied with the season, supporting entrainment. Light was generally avoided at levels of civil twilight (ca 1 lux) and higher. An individual animal tracked the lower reaches of dusk and/or dawn in a manner accountable by the phase-responses required to correct for tau_{DD}, and the constraint imposed by night length. Under the long winter night, for example, an animal with tau <24 h would self-expose primarily to dusk (yielding a phase delay), while one with tau >24 h would encounter dawn (yielding a phase advance). Across the seasons, nighttime was spent with active shuttling *above* and *below*. Behavior *above* was constrained in summer due to short night length, with the animal "forced" to self-expose to both dusk and dawn. The initiation of nocturnal feeding and drinking, however, always occurred *below* in summer, several hours before dusk. By allowing the behavior to spill over *below*, alpha duration was conserved (ca 12 h) across the year. Given the changing dawn time horizon, however, in which behavior *above* could not outlast rather low twilight levels, animals with tau >24 h showed a phase-delay of alpha in winter (late dawn) relative to summer (early dawn).

This experiment demonstrates that when rats are given the opportunity to sample a naturalistic, cyclic lighting environment, various predictable self-generated skeleton photoperiods result, with maximum light level and total daily duration far below that usually used to entrain circadian rhythms to LD cycles in the laboratory or colony room. *[Research supported by NIMH Grants KO2 MH00461 and R43 MH40584.]*

20 DISK-SHEDDING AND DOPAMINE RHYTHMS UNDER SIMULATED DAWN AND DUSK. R.A. Bush, Ch.E. Remé, M.Terman*, A.Malnoe**, University Eye Clinic, Zuerich, Switzerland, *New York State Psychiatric Institute, New York, **Nestlé Research Center, Lausanne, Switzerland

Gradual twilight transitions at dawn and dusk may be important for the entrainment of circadian rhythms. The circadian rhythms of rod disk-shedding and retinal dopamine , which are thought to be functionally related, were analyzed in rats reared from birth under cyclic light with natural dawn and dusk transitions simulated by a computer-controlled illuminator. Another group was reared under cyclic light with abrupt transition (12h on:12h off) usually present in the laboratory. Maximum (15 lux) and minimum (darkness) intensities were reached at the same times in the two conditions. Phagosomes were counted in the superior and inferior central retina of one eye, DOPAC content, an indicator of dopamine utilization, was measured in the retina of the other eye of rats sacrificed at six different time points of the light-dark cycle. One hr after light onset, the same peak-number of phagosomes was observed in both conditions. However, shedding under the illuminator was triggered at an intensity as low as $5x10^{-4}$ uW/cm^2 (0.002 lux) and declined at a slower rate thus resulting in a significantly earlier and broader peak relative to the abrupt light onset condition. Therefore, disk-shedding is triggered by extremely low levels of light but progresses independent of stimulus intensity up to 15 lux. The rhythm of retinal DOPAC content was correlated with disk-shedding in both groups ($r=0.85$), but was not significantly shifted by the presence of gradual dawn, implying a lower light sensitivity than disk-shedding.

21 **MODULATION OF MELATONIN AND UTERINE CONTRACTILE RHYTHMS BY PHOTOPERIOD IN THE PREGNANT RHESUS MACAQUE.** C.A. Ducsay and S.M. Yellon.
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In a variety of species, photoperiod influences the time of day of birth. In the rhesus macaque, nocturnal rhythms in uterine contractility during late gestation are paralleled by 24h patterns in both maternal and fetal plasma steroids. The present study tested the hypothesis that photoperiod phase entrains the 24h pattern in myometrial contractility and the circadian melatonin rhythm in the mother. Seven rhesus macaques, acclimated to 12 h of light per day (**0700** to **1900h**) underwent surgery between 120 and 126 days gestation (term = 166 days) for implantation of maternal vascular and amniotic fluid catheters. Intrauterine pressure was continuously recorded throughout the study. Five to seven days later, blood samples were collected at 3h intervals over a 24h period beginning at 0900h, 2h after lights on, and 0.5h before and after lights on and off. A characteristic nocturnal uterine activity rhythm was observed; contractile events peaked at 2400h compared to the nadir at 1400h ($p < 0.05$ ANOVA). Daytime plasma melatonin levels averaged 31 ± 6 pg/ml compared to the peak of 60 ± 6 pg/ml at 2400h ($p < 0.05$). The minimum duration of the melatonin rise approximated 5h. Photoperiod was then shifted 11 h (lights on from **2000** to **0800h**). After 7 days, blood samples were again collected as described above beginning 2h after lights on at 2200h, and the sampling protocol was repeated at weekly intervals until delivery (15 bleeds in 5 animals). After 7 days of reversed photoperiod, the peak in the uterine activity shifted to 1300h while the nadir occurred at 2000h ($p < 0.05$). Melatonin concentrations increased from a mean of 22 ± 4 pg/ml during the light phase to a nighttime peak of 60 ± 5 pg/ml ($p < 0.01$) at 1300h; this rise began 0.5h after lights off and continued for approximately 11h. A similar phase relationship to lights off was maintained after reversed photoperiod for both myometrial activity and circulating melatonin rhythms. Furthermore, of the animals in the study that went to term ($n = 3$), the normal time of day of delivery was reversed. These findings indicate that photoperiod entrains rhythms in uterine contractile activity and melatonin secretion during late gestation. These rhythms, and 24h patterns of other hormones, appear to play a role in the timing of parturition. (NIH HD22865 and LLU Seed Grant).

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DO NMDA RECEPTORS MEDIATE THE EFFECTS OF LIGHT ON CIRCADIAN BEHAVIOR IN THE HAMSTER?

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We report here the results of experiments designed to evaluate whether a specific NMDA receptor antagonist, (+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate (MK-801), blocks the phase shifting effects of light on the circadian rhythm of wheel-running activity in golden hamsters. Intraperitoneal administration of (+)MK-801 produced a dose dependent blockade of both light-induced phase advances and delays. The effect was stereoselective and treatment with related compounds, phenylcyclidine and ketamine, also blocked light-induced phase shifts. MK-801, by itself, did not cause any consistent effect on the phase of the rhythm. These data, coupled with previous findings, indicate that excitatory amino acid receptors play an important role in the transmission of light information from the retina to the circadian system.

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AMPLITUDE OF CIRCADIAN PACEMAKER AND THE PHOTOPERIODIC RESPONSE. Colin S. Pittendrigh, University of Arizona, Tucson, AZ 85721.

Evidence from the kinetics of rhythm phase-shifts indicates that the amplitude, as distinct from its period, of the Drosophila eclosion pacemaker is temperature dependent. As the amplitude of the pacemaker's motion is increased, the amplitude of the Phase Response Curve (PRC) for a given light-pulse decreases. It is argued that this is the cause of the latitudinal cline in D. auraria's PRCs for the eclosion pacemaker, and that the pacemaker's sensing of photoperiod is a function of its amplitude declining as photoperiod increases. Latitude and temperature effects on the photoperiodic response curves of D. auraria have the same foundation--i.e. change in average amplitude.

24 DIAPAUSE INDUCTION: ARE LOW TEMPERATURES FUNCTIONALLY EQUIVALENT TO LIGHT? G.T.Wassmer, W.Cain, E.D.DeAngelo, and S.D.Skopik School of Life and Health Sciences, U. of Delaware, Newark, DE 19716

The induction of larval diapause in the European corn borer (Ostrinia nubilalis) can be controlled by photoperiodic and/or thermoperiodic signals. Light-dark (LD) cycles consisting of 12 hours of light alternating with 12 hours of dark (LD 12:12) at a constant temperature effect maximal diapause. Thermoperiods composed of 12 hours at 25°C and 12 hours at 15°C (CW 12:12; C=cold, W=warm) in constant darkness also bring about maximal diapause. When these two diapause inducing regimes are combined such that the cryophase coincides with either the scotophase or the photophase maximal diapause is once again effected. However, Skopik et al. (1986, J. Biol. Rhythms 1(2):145-150) made the surprising observation that when a thermoperiod in which the cryophase was 4°C was combined with LD 12:12 with the cryophase coinciding with the scotophase diapause was not induced. Alternatively, when the cryophase occured during the photophase all of the larvae entered diapause. The authors hypothesized that the cryophase may have been functionally equivalent to photophase with respect to photoperiodic time measurement.

In order to test this hypothesis we exposed groups of insects to CW 16:12 (C=4°C, W=25°C) in constant dark, CW 12:16 in constant dark, LD 16:12 at 25°C, or LD 12:16 at 25°C. We predicted that if the cryophase is equivalent to the photophase then the response to LD 12:16 should equal that to CW 12:16 (low diapause) and LD 16:12 should be the same as CW 16:12 (high diapause). Alternatively, if the cryophase is equivalent to the scotophase then the opposite response should be observed, that is LD 12:16 and CW 16:12 should bring about the same response (low diapause) and both LD 16:12 and CW 12:16 should result in high diapause. Our results showed clearly that the first alternative was correct and that a cryophase of 4°C appears to be functionally equivalent to a photophase at 25°C.

25 EFFECT OF ENPROSTIL, A PROSTAGLANDIN E_2 ANALOGUE, ON THE CIRCADIAN RHYTHM IN DNA SYNTHESIS IN MOUSE GUT. N.H. Rubin, P.R. Laraby, J.B. Field, P.L. Rayford*, C.M. Townsend, Jr., J.C. Thompson. Department of Surgery, The University of Texas Medical Branch, Galveston, Texas, and *Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Cell proliferation in the gastrointestinal tract is characterized by endogenous circadian variation. Several gut hormones are trophic or antitrophic to gut mucosa. Gastrin stimulates DNA synthesis in the stomach; however, we have reported that in chronobiologically-designed studies, pentagastrin stimulated DNA synthesis only during a span of 8-12 h, when DNA synthesis was increasing in control mice, due to the circadian rhythm, and had no effect at other times. Agents that alter release of gastrin may affect its trophic action. Enprostil, a long-lasting prostaglandin E_2 analogue, inhibits meal-stimulated gastrin release. We observed the effect of enprostil on circadian rhythms in DNA synthesis in mouse gut. Two groups of ad lib fed C57BL mice (female, 8 months old) were acclimated to a 12:12 light:dark cycle. Group A were given enprostil intraperitoneally (IP, 50 μg/kg, t.i.d., 5 days); group B received diluent only. On Day 6, mice were killed, one subgroup from groups A and B every 4 h for 24 h. Injections were continued t.i.d. in the remaining subgroups. Each mouse received tritiated thymidine (^{3}HTdR, IP, 25 μCi) 30 min before it was killed by cervical dislocation, and uptake of ^{3}HTdR was measured. Results are reported as counts per minute (CPM) per μg of DNA and were analyzed by ANOVA and Student's T-test. We found significant rhythms in DNA synthesis in stomach and colon. DNA synthesis was significantly higher in enprostil-treated mice. A biphasic curve characterized uptake of ^{3}HTdR in colons from both groups. In stomach, the highest activity in controls was at 0500 h (3989 CPM/μg DNA), the lowest at 1700 h (1212 CPM/μg DNA). The highest activity in enprostil-treated mice was at 0500 h (5872 CPM/μg DNA); the lowest, 1300 h (3345 CPM/μg DNA The same drug regimen given at different times in the circadian cycle yielded significantly different results. In intact animals, studies on the effects of various stimulators or inhibitors of DNA synthesis should be time-qualified.

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LIGHT INDUCES C-FOS mRNA IN THE SUPRACHIASMATIC NUCLEUS OF HAMSTER. **Jon M. Kornhauser, Dwight E. Nelson, Kelly E. Mayo, and Joseph S. Takahashi. Department of Biochemistry, Molecular Biology and Cell Biology, and Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.**

The product of the c-fos gene is a component of the AP-1 transcription factor, which can regulate the expression of specific target genes. Rusak and Robertson, Schwartz and Aronin, and Earnest et al. (Soc. Neurosci. Abst., 1989) have shown that c-fos immunoreactivity in the suprachiasmatic nucleus (SCN) of rodents is elevated by light. To begin investigating whether fos is regulated transcriptionally by light, we have determined levels of c-fos mRNA in the hamster SCN. "Immediate-early genes" such as fos could potentially mediate changes in gene expression involved in the entrainment of the circadian clock to environmental light conditions.

Golden hamsters maintained on a 14:10 light/dark cycle were exposed to a monochromatic light pulse at circadian time (CT) 19; control animals remained in the dark. After various times of light duration, brains were sectioned for in situ hybridization. Using a complementary RNA probe for c-fos, we found no detectable hybridization in dark control animals. Light-pulsed hamsters displayed a strong induction of c-fos mRNA in the region of the SCN, with most of the signal localized to the ventrolateral portion of the nucleus. No signal was detected in other retinorecipient areas of the brain. A significant induction was seen after a 15 min light pulse, and the maximal level was observed after 30 min of light, after which the signal declined despite continued light exposure. These results invite speculation about a possible role for fos in entrainment. Further analysis of the effects of light in altering gene expression may provide insight into the cellular mechanisms of entrainment of the circadian clock.

27 **PHASE DEPENDENT INDUCTION OF THE PROTO-ONCOGENE *fos* (c-*fos*) IN HAMSTER SUPRACHIASMATIC NUCLEUS (SCN).** J.SERVIERE, G.GENDROT, D.MENETREY*, F.XAVIER & J.de POMMERY*. INRA 78350 Jouy en Josas, *INSERM U161 Paris, FRANCE.

In mammals, entrainment to light/dark cycle (L:D) and generation of circadian rhythms (CR) are controlled by a dominant endogeneous pacemaker located within SCN. Under constant darkness, the CR of locomotor activity free-runs and can be shifted by light pulses in a phase dependent manner (phase response curve, PRC). Since c-*fos* proto oncogene expression is one of the immediate and temporary intracellular responses to external stimuli inducing long lasting changes in neurons, we sought to study the fluctuations of flash-induced c-*fos* expression over a circadian cycle.

Following entrainment to a 16L:8D cycle (circadian time, CT12=L:D transition), male Syrian hamsters were kept 24h in darkness, exposed to a 4min sequence of bright flashes (6 per min) at CT 00, 05, 13, 18 and perfused 45min later for determination of immunoreactivity to Fos (Fos-ir, polyclonal antibody to $Fos_{total\ protein}$). Non-stimulated animals had virtually no basal Fos-ir in SCN. CT18 flash-exposed animals were the only ones to exhibit strong Fos-ir in ventro-lateral SCN (area of retinohypothalamic tract terminals), dorsal and lateral areas surrounding SCN, anterior and lateral hypothalamic areas, paraventricular nuclei of the thalamus. Expression of c-*fos* mRNA in different brain areas of flash-exposed and control animals at the same circadian times is under current study by Northern and dot blot analyses.

This phase dependent c-*fos* expression precisely parallels the behaviourally expressed PRC and could be the coupling factor between immediate electrical activation of SCN by light and long term transcriptional events mediating entrainment of circadian rhythms.

28 MELATONIN INDUCES c-FOS EXPRESSION IN THE SUPRACHIASMATIC NUCLEI. T.S. Kilduff, H. B. Landel, G. S. Nagy, H.C. Heller, and W.C. Dement. Depts. of Psychiatry and Biological Sciences, Stanford University, Stanford, CA 94305.

Fos, an inducible DNA-binding protein, belongs to a class of transient "immediate-early" genes that has been proposed to couple short-term membrane events to long-term changes in gene transcription. Given that a high affinity melatonin receptor has been described in the suprachiasmatic nuclei (SCN) and that melatonin has a biphasic effect on metabolic activity in the SCN, we have investigated whether melatonin administration affects Fos expression in the SCN.

Male Wistar rats, entrained to LD 12:12, were released into constant darkness and given subcutaneous injections of either melatonin (10 or 100ug/kg) or vehicle under dim red light. After a 1 or 2 h incubation, animals were anesthetized and perfused with a 4% PFO solution. Brains were post-fixed in 4% PFO at 4°C for 4 h, washed in PBS, and vibratome sliced at 100u. Sections were labelled immunohistochemically using a monoclonal antibody raised to residues 4-17 of the Fos protein (Microbiological Associates, Bethesda, MD).

Administration of 100ug/kg melatonin at CT22 resulted in robust expression of Fos immunoreactivity in the nuclei of SCN cells. The same concentration of melatonin was ineffective at inducing Fos immunoreactivity in the SCN at CT10, as was the vehicle at both time points. A reduced effect was achieved by injecting 10ug/kg melatonin at CT22, with fewer labelled cells concentrated in the ventral SCN. There was no endogenous Fos expression in the SCN at either CT10 or CT22, and Fos expression was not induced in the intergeniculate leaflet. These results indicate that melatonin influences the transcriptional machinery of cells within the SCN in a circadian phase-dependent manner that corresponds to the times at which it also induces maximal inhibition (CT10) and elevation (CT22) of metabolic activity in the SCN. Changes in gene transcription indicated by c-fos or other transcriptional activators may provide insights into the molecular machinery of the biological clock (Supported in part by a grant from the Upjohn Company).

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CHARACTERIZATION OF MELATONIN RECEPTORS IN THE RAT SUPRACHIASMATIC NUCLEI AND AREA POSTREMA: AFFINITY SHIFTS WITH GUANINE NUCLEOTIDES AND MONOVALENT CATIONS

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An iodinated melatonin analog, 2-^{125}I-melatonin (2-^{125}I-MT), has proven to be the ligand of choice in revealing putative receptors for the pineal indole in the brain and retina of various species. In the present study, we have characterized 2-^{125}I-MT binding sites in the rat suprachiasmatic nuclei (SCN) and area postrema (AP) by using quantitative autoradiography in vitro.

Saturation studies revealed high affinity binding sites in both areas (K_d around 50 pM) with different binding capacities (B_{max} 15 and 30 fmol/mg protein for the SCN and AP, respectively), when incubations were carried out in 50 mM TRIS-HCl-buffer, pH 7.40. In thin layer chromatography, bound and free radioactivity comigrated with unlabeled 2-I-MT suggesting that the integrity of the ligand was maintained. Association/dissociation experiments demonstrated reversibility of 2-^{125}I-MT binding in both areas. The K_ds calculated from the kinetic experiments closely agreed with those obtained from the saturation studies. In both areas, the binding sites were specific for melatonin but not for its precursor N-acetylserotonin or other related indoles.

Micromolar concentrations of guanine (but not adenine) nucleotides dose-dependently inhibited agonist binding at 22 °C by decreasing the K_d of the receptors both in the SCN and AP. Shortening of the washing step in autoradiography allowed the demonstration of an additional lower affinity binding site in the SCN. The above data suggest coupling of these melatonin receptors to guanine nucleotide binding regulatory protein(s) (G-proteins). Furthermore, monovalent cations (Na^+ and Li^+) dose-dependently inhibited agonist binding by decreasing the K_d of these receptors. This effect was more pronounced in the SCN than in the AP and readily occured at physiologically relevant Na^+-ion concentrations. This suggests that the affinities obtained in hypotonic TRIS-buffer might be overestimated. Addition of divalent cations (Ca^{++} and Mg^{++}) into incubation buffer had little (SCN) or no (AP) detectable effect on binding affinity.

In summary, our data demonstrate putative melatonin receptors in the rat SCN and AP. Manipulation of these receptors in vitro revealed the presence of high and low affinity states, a typical behavior for cell membrane receptors that are known to communicate through G-proteins. The data agree with recent evidence on melatonin receptor coupling to G-proteins in sheep/hamster pars tuberalis and in lizard brain.

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THE AMPLIFICATION OF *DROSOPHILA per* GENE HOMOLOGS FROM RAT AND MOUSE BRAIN cDNA LIBRARIES

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One of the two clock genes cloned to date is the *per* gene of *Drosophila melanogaster*. Recently it has been shown that an antibody to a small (14 amino acid) peptide-S derived from the *per* protein specifically stains cross-reactive antigens in the eyes of the molluscs Aplysia and Bulla. These results imply a certain degree of evolutionary conservation of the *per* protein.

Based on those results, we have used a 1.9 kb fragment of the *per* gene, which does not contain the Thr-Gly repeats, as a probe on Southern blots of various mammalian genomic DNAs. Chinese hamster ovary (CHO), mouse, Syrian hamster and human genomic DNAs digested with BamH1 exhibited bands of approximately 5 kb under stringent washing conditions (0.2x SSC).

The degenerate primer PCR method was used to amplify the putative *per* gene homologs from two mammalian cDNA libraries. One was a λ-gt11 rat brain cDNA library and the other was constructed from a mouse anterior hypothalamus in λ-ZAP vector. Two sets of primers were devised based on the *per* protein deduced amino acid sequence. The 3' end primer was based either directly on the peptide-S or immediately flanking this region downstream. Each of the three 5' end primers was designed to span each of the three major *per* mutations. Only one set spanning the per^0 mutation gave positive signals when the PCR products were probed with the 1.9 kb *per* fragment on Southern blots. The probe signal was much stronger when the 3' end primer flanking the peptide-S was used. Two bands were identified, one 0.5 kb (the distance between the primers) and one approximately 0.6 kb. The 0.6 kb band was more intense, when amplified from the rat brain library. Experiments are now in progress to subclone and sequence these *per* homologs. These results provide evidence for the conservation and expression of the *per* gene homologous sequences in mammalian brain and in particular in the SCN.

31 CYCLIC AMP, CELL DIVISION CYCLES, AND CIRCADIAN OSCILLATORS IN EUGLENA. Leland N. Edmunds and Isabelle A. Carré. Division of Biological Sciences, State University of New York, Stony Brook, NY 11794.

Oscillations in adenosine 3',5'-cyclic monophosphate (cAMP) level have been proposed to be part of the biochemical feed-back loop(s) believed to underlie circadian rhythmicity and may be especially involved in the control of the cell division cycle (CDC) by the circadian clock. There is also evidence that a transient rise and ensuing fall of cAMP level are necessary for the initiation of DNA synthesis, and that a second cAMP surge is correlated with the onset of mitosis. A clock-controlled variation of the cAMP level (that is, the periodic repetition of a cAMP signal) then may participate in the 'gating' of cell division to a certain phase of the circadian period. This possibility has been examined for a cellular circadian oscillator in synchronously dividing or nondividing cultures of the photosynthesis-deficient ZC mutant of the alga Euglena gracilis Klebs (Z).

We have demonstrated bimodal, circadian changes of cAMP level in division-phased populations in LD:12,12. This rhythm in cAMP level persisted in dividing cultures maintained in DD. These variations, however, appear to be under the control of the circadian oscillator rather than to be cell cycle-dependent, since they continued, independently of the CDC, in cultures that had reached the stationary phase of growth. The rhythm in nondividing cultures could be phase-shifted by light signals, in a manner that could be predicted from the PRC previously obtained for the perturbation of the division rhythm by light pulses. Although pulses of cAMP (demonstrated to enter the cells from the medium in a dose-dependent manner) do not appear to generate significant steady-state $\Delta\phi$s of the division rhythm in Euglena, they do seem to be able to cause either an acceleration or a lengthening of the CDC depending on the phase of the CDC when the drug is given: injection of cAMP (0.5 mM) in the medium at CT06-08 (corresponding to the end of G_1 phase) resulted in delay of the following synchronous division burst, while the same pulse given at CT 21-23 (corresponding to the early G_1 phase) caused advanced division of some cells so that division appeared desynchronized during the next 24 h. These results suggest that cAMP is an element of the coupling pathway for the control of the CDC by the circadian oscillator.

32 MONOCLONAL ANTIBODIES TO A UNIQUE ANTIGEN IN NEUROSECRETORY CELLS OF A CIRCADIAN ORGAN, THE *APLYSIA* EYE

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The eye of *Aplysia* contains a circadian oscillator which is thought to reside in the neurosecretory output neurons of the eye. We have produced two monoclonal antibodies 8ON7 and NSC7, resulting from different fusions, which specifically recognize neurosecretory cells (NSCs) in tissue sections of the *Aplysia* eye. Quantitative immunofluorescent analysis of *Aplysia* eye cell cultures, using serial dilutions of 8ON7 and NSC7, further demonstrates the specificity of these antibodies to mono- and bipolar neurons (putative NSCs). Photoreceptors and pigment support cells show near background fluorescence at all dilutions, while NSCs fluoresce increasingly with higher antibody concentrations.

By Western blot analysis, 8ON7 and NSC7 demonstrate immunoreactivity against a major eye specific protein in *Aplysia*. Two-dimensional gel analysis indicates that this protein is acidic and has a molecular weight of 62-64 kD. These antibodies show no immunoreactivity by Western blot with either the soluble or the membrane fractions of any other *Aplysia* tissue tested (ganglia, pedal nerve, heart and skin).

The physiologic relationship of this protein to the neurosecretory and/or circadian functions of the eye is currently under investigation.

33 LOCOMOTOR ACTIVITY ACCELERATES RE-ENTRAINMENT OF THE CIRCADIAN TEMPERATURE RHYTHM TO A DELAYED SLEEP-WAKE CYCLE IN MAN.

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In previous studies with hamsters (Mesocricetus auratus) it was found that apart from other non-photic stimuli single locomotor activity pulses scheduled at appropriate times during the circadian cycle can induce phase-shifts and alter the rate of adjustment to new light-dark cycles (1),(2).

The aim of the present pilot study was to determine whether a single activity pulse is capable of accelerating the adaptation of the human core temperature rhythm to abrupt shifts of the sleep-wake cycle such as those involved in shift work.

Three volunteers were tested under their usual working conditions, experiencing a 6-8h delay in their sleep-wake cycle by switching from day- to night-shift. Two of them performed on one or two additional change of shifts single locomotor activity pulses of 90 min on the first night of the night-shift. Rectal temperature and locomotor activity were recorded continuously by a portable data aquisition system for 12-15days, comprising 5-7days of day-shift followed by 7days of night-shift. Period length, circadian phase and amplitude were assessed with time series analyses including periodograms, and complex demodulations.

Single activity pulses scheduled at times when a delay of the cycle would be expected caused a faster re-entrainment by phase jumps. One volunteer, doing exercises on an ergometer from 9:30 -11:00 pm, showed a phase jump of 4-5h, the other volunteer, jogging from 10:00-11:30 pm showed a phase jump of 2h but on another occasion showed a phase jump of 4h after exercising on an ergometer from 0:00-1:30 am. Under night-shift conditions without activity pulses the temperature rhythm re-entrained with transient cycles of 24,5-25,0h in all of the three volunteers.

These results indicate that activity can enhance the rate of re-entrainment to phase shifted sleep-wake cycles and would act as an effective Zeitgeber for the temperature rhythm. The magnitude of the phase jumps is likely to depend on the circadian time the activity pulse is given at.

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34 PHASE-SHIFTING EFFECT OF INDIRECT (CEILING-MOUNTED) BRIGHT LIGHT EXPOSURE ON THE HUMAN CIRCADIAN PACEMAKER.

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In most clinical and research phototherapy reported to date, a vertical source of bright light has been used to induce phase shifts of the human circadian system. While direct exposure to vertical light sources have proven effective, they require the subject/patient to remain in a limited area and direct their angle of gaze toward the light source. The present study evaluated whether indirect exposure to bright light from a ceiling-mounted source could induce phase shifts of the endogenous circadian temperature rhythm comparable to those achieved by direct light exposure.

A stimulus consisting of 3 cycles of exposure to a repeated daily illuminance pattern that included indirect exposure to bright light (5 h per cycle of ~10,000 lux), room light (16 h per cycle of ~150 lux), and darkness (8 h of <2 lux per cycle) was applied to 3 male subjects ages 18-25. The protocol for each was designed to allow comparison with previously conducted trials of direct exposure to bright light. Endogenous circadian phase was evaluated before and after exposure to the 3-cycles of light using the constant routine technique. The midpoint of the overall light stimulus was scheduled to occur near the steepest part of the phase response curve.

We found that exposure to this 3-cycle stimulus induced phase shifts ranging from 5 to 12 hours. Each of these results was within the 95% confidence interval for the mean of the final circadian phase of the light stimulus derived from a statistical evaluation of our model fit to the phase transition curve we previously reported for 45 trials of direct light exposure. The results suggest that indirect bright light exposure can induce phase shifts which are comparable to those achieved by direct bright light exposure, including the complete inversions of circadian phase required to achieve Type 0 resetting.

35 **INTERPRETATION OF THE CIRCADIAN PATTERN OF MELATONIN SERUM CONCENTRATIONS IN WOMEN: WHICH METHOD IS BEST?** Sarah L. Berga, Gail A. Laughlin, Michael L. Johnson, Anne B. Loucks and Samuel S.C. Yen, The University of Pittsburgh, The University of Virginia, and the University of California, San Diego

Melatonin concentrations in the peripheral circulation of adult women exposed to a natural photoperiod begin to rise above the threshold of detection around dusk, peak in the middle of the dark phase, and reach undetectable levels again around dawn. Daytime levels generally do not exceed the threshold of detection. The following parameters have been used to describe the idealized circadian profile of melatonin in humans: onset (ON), offset (OFF), duration (DURA), midpoint (MPT), and magnitude (MAG). ON has been defined as the first point that exceeds the daytime baseline that is followed by two other points of equal or greater value, OFF as the first point at or below the baseline that is followed by two points of equal or lesser value, DURA as the difference between ON and OFF, MPT as ON + OFF/2 or the time of the single highest value, MAG as the single highest or mean of the two or three highest value(s) between ON and OFF. Using the operational definitions (OP), there are three independent variables (ON, OFF, and MAG) and the shape of the nocturnal pattern is not specified. Traditionally the circadian pattern of a hormone has been described in terms of the circular derivatives acrophase (ACRO), amplitude (AMP), nadir, and mesor. Only two of these descriptors (ACRO and AMP) apply to the melatonin configuration. By definition, ACRO and AMP are derived after fitting the peripheral concentrations to a curve. The commonly used cosine curve is inadequate for melatonin because its pattern has no nadir and thus AMP is grossly underestimated. Also, ON and OFF are not identified. In an attempt to better describe the pattern of melatonin levels in humans, we sought to develop a curve-fitting program which would identify ON, OFF, DURA, MPT (ACRO), and MAG (AMP).

Blood samples were obtained at 30 min intervals from 1700-1000h and at 60 min intervals during the day in seven women with athletic amenorrhea and seven women with regular menstrual cycles. Melatonin concentrations were determined by a sensitive and specific double-antibody RIA. To estimate ON, OFF, and DURA, three methods were used. The data were fit to an asymmetric triangle (TRI) and an ellipse (ELL) using a weighted least squares analysis. Onset and offset were determined also by deconvolution (DEC) in order to estimate secretory events. The results of the three methods were compared to those obtained using OP. Three methods were used to estimate ACRO (MPT) and AMP (MAG): (1) TRI; (2) ELL; and cosinor (COS) and the results were compared to those generated by OP.

The results generated by OP were closest to the actual data points because MAG, ON, and OFF were independent of one another. Distortions of ON, OFF, DURA, or MAG were caused by curve-fitting. With TRI, the degree of distortion depended on MAG. The higher the rise, the less the distortion of ON and therefore DURA. Although MAG and OFF were well estimated using TRI, the results were similar to those obtained with OP and thus TRI offered no clear advantage. ELL identified ON well, but tended to underestimate MAG, especially when MAG was high. As expected, COS grossly underestimated AMP (MAG). The "secretory" OFF identified by DEC was earlier because it accounted for the delay introduced by clearance time. DEC and OP gave similar values for ON. All methods gave similar results for ACRO (MPT). We conclude that OP provides the best overall interpretation of the 24-h pattern of melatonin and that DEC can be used to estimate "secretory" OFF when necessary.

36 EFFECT OF PHOTOTHERAPY ON MOOD DISORDERS

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Previous reseaches have indicated that exposure to bright artificial light can benefit patients with seasonal affective disorder (SAD). However, the effects of the bright light have been examined only in a small number of nonseasonally depressed individuals. In the present study, we performed the bright light therapy either in the morning or in the evening to the 15 depressed patients and 6 normal controls. Phototherapy (2500 lux) was administered for 2 hours, either between 0600 and 0800 or between 1800 and 2000, for 7 consecutive days. Both before and after these therapies, we measured the deep body temperature from the skin surface every 2 hours for 2 days to examine the effects of the light on the circadian rhythm. In all patients, the scores of the Hamilton Rating Scale for Depression decreased after the bright light treatment. Nine of 10 patients, who were exposed to the bright light in the morning, advanced their acrophases of body temperature rhythm. Three of 5 patients, who were exposed in the evening, advanced their acrophases while the other 2 patients delayed them. There was no relationship between the extent of advance or delay and that of improvement of depression. No consistent changes were found in the amplitude and the mesor of body temperature rhythm after both morning and evening treatments.

37 **ULTRADIAN RHYTHMS IN EVENT-RELATED CORTICAL ACTIVITY AND PERFORMANCE**

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Event-related potentials (ERPs) are noninvasive measures of central nervous system activity that provide information about attention, cognitive processing, and arousal. They can be seen as positive and negative-going deflections that are generated by component neuronal processes. Diurnal and circadian variations have been reported in ERPs. This study assessed ultradian rhythmicity in auditory ERP components and their relationship to rhythmicity in performance. Eight subjects were tested every 15 min for 8 hrs. A constant routine was maintained. ERPs were evoked by a series of target (2000 Hz, p=.10) and non-target (1000 Hz, p=.90) tones (60 dB, ISI=1 sec). Recordings were from Cz, Fz and Pz leads; only the results of Cz are reported here. Subjects were instructed to make a finger-lift response to target tones, and reaction time (RT) was recorded. Time series for latency and amplitude measures of ERP components and for RT were visually inspected for rhythmicity, then detrended and analyzed by power spectral analysis. Periodicity in the range of 60-120 min was demonstrated by all subjects in one or more ERP measures. Activity at slower frequencies confirmed diurnal variations reported by other investigators. RT demonstrated rhythmicity with an average period of 100 min. Cross-correlational analysis indicated ERPs and RT were related. Symmetrical cross-correlation functions were seen in all subjects, often in several measures. However, there were wide interindividual differences in which ERP measures best demonstrated the relationship, as well as in period and phase. This study indicates that a portion of the variation typically observed in ERPs may be due to the influence of one or more biological oscillators. The relationship between rhythmicity in ERPs and RT suggests that ERPs may serve as a biological marker of time-related changes in performance.

38 DIURNAL VARIATION IN N100-P200 COMPLEX OF THE VISUAL EVOKED POTENTIAL

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The N100-P200 complex of the evoked potential (EP) is considered an index of attentional processes related to stimulus encoding. Prior research suggested that the auditory N100-P200 displays a diurnal variation (afternoon superiority) mapping stimulus detection efficiency, although contradictory findings have been reported. Furthermore, no study has adequately assessed N100-P200 diurnal variations for the visual modality. The present analysis addressed the latter issue by assessing N100-P200 changes across a broad time span with frequent sampling across the day and at different scalp sites. A Latin square design was used to control amplitude changes due to repeated testing (habituation).

Eleven female subjects were tested on a visual semantic categorization task every two hours from 0900 to 2100 (first test time staggered across subjects). They indicated via key press whether a word presented on a CRT represented an instance of the previously presented category. Evoked potentials were recorded and averaged separately for time of day (0900, 1100, 1300, 1500, 1700, 1900, and 2100 hrs), word instance (positive vs. negative) and scalp site (Fz, Cz and Pz). The N100-P200 complex was scored as the amplitude difference between peaks of the negative-positive deflection beginning approximately 100 ms post-stimulus (N100 peak).

A three-way within-subjects' ANOVA for Time of Day, Scalp Site and Word Instance performed on N100-P200 amplitude revealed a significant main effect for Time of Day ($F[6,60]=4.22, p<.05$). Trend analyses performed on mean N100-P200 amplitude across Blocks (collapsed across Scalp Site and Word Instance) revealed a significant linear component ($F[1,10]=37.68$, $p<.05$) with amplitude increasing across the day. A significant main effect for Site also was found ($F[2,20]=5.22, p<.05$); amplitude was higher at Cz (-5.3 uV) than at Pz (-4.8 uV) or Fz (-4.7 uV). Pz and Fz were not significantly different. No other effects were significant. Categorization errors did not vary across the day ($p>.05$).

Results of the present study indicate that N100-P200 amplitude of the visual EP increases across the day. This pattern confirms previous reports of higher visual N100-P200 in the late afternoon for "Evening Type" subjects and identifies the pattern across a broad timespan. Interestingly, performance (categorization errors) did not vary with N100-P200 amplitude, suggesting that for some tasks, diurnal variations in stimulus encoding processes (as indexed by N100-P200), do not affect performance, particularly if the stimuli are easily detected.

39 RHYTHMIC ORGANIZATION OF SPONTANEOUS MOVEMENTS IN PREMATURE INFANTS LESS THAN 34 WEEKS GESTATIONAL AGE. Marie J. Hayes, Savitri P. Kumar, Lonnie Plante, and Maria Delivoria-Papadopoulos, Dep't of Pediatrics, Univ. of Pennsylvania, and Dep't of Psychology, Univ. of Maine.

Previous research indicates that fetal motility is temporally organized in short ultradian cycles in both humans and animals. In the present study, the spontaneous motor activity of ten healthy premature infants between 25 and 34 weeks gestational age was monitored using a pressure-sensitive transducer placed under the infant's head and torso. Transducer output was sampled every 0.5 sec by a personal computer and interface system. Subjects were monitored during a single two to three hr session, with the exception of three infants who were studied on a second occassion to assess intrasubject reliability. Sessions were timed to begin at least one hour after feeding for intragastrically-fed infants, who were generally on a 4-hr feeding schedule. Data were analyzed for periodicity using Fourier analysis (FFT) and least-squares variance spectra. These analyses have demonstrated the presence of several prominent cycles in the activity data. Significant spectral peaks were found at periods of approximately 30 min, 15 min and 10 min, and most peaks were detected by both analyses. These results corroborate in premature human infants previous findings for fetal activity rhythms. Our results suggest that spontaneous motility rhythms are an endogenous property of early central nervous system function, expressed similarly both *in utero* and *ex utero*.

40 A WAVEFORM-INDEPENDENT DECONVOLUTION TECHNIQUE TO ANALYZE *IN VIVO* NEUROHORMONE SECRETION. M.L. Johnson*, A.E. Lassiter*, and J.D. Veldhuis, Interdisciplinary Graduate Biophysics Program, Division of Endocrinology and Metabolism, Departments of Pharmacology and Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908.

We have developed a waveform-independent deconvolution method designed to describe quantitatively the secretory behavior of (neuro)endocrine glands *in vivo*. This procedure estimates positive real non-zero values of secretion and defines statistical confidence limits for each sample (point) observation based upon the dose-dependent experimental uncertainty in the hormone measurements and the statistical error associated with the hormone half-life of interest. The ill-posed nature of some discrete deconvolution algorithms is obviated by formulating a family of convolution integrals to describe the sample hormone concentrations over time without a time differential in the denominator of any expression. Application of this new, waveform-independent deconvolution methodology to physiological endocrine time series (LH, FSH, PRL, TSH, GH, ACTH, cortisol, and insulin) revealed a predominantly burst-like mode of glandular secretion, in which secretory events had finite non-zero durations. Corresponding estimates of endogenous hormone secretion rates were physiologically reasonable. Moreover, the fitted variance for this technique was less than eight percent of the mean hormone concentration, which approximates the experimental uncertainty inherent in the measurement system. We conclude that a waveform-independent, quantitative, and relatively well-behaved deconvolution method of evaluating hormone secretion *in vivo* yields good estimates of endogenous hormone secretory rates; provides an excellent fit of observed hormone concentration time series; and unmasks a distinctly burst-like mode of hormone secretion by the anterior pituitary gland, adrenal zona fasciculata, and pancreatic beta cells.

41 EPISODIC RENIN RELEASE DURING SLEEP IN SOME PATHOLOGICAL CASES.

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In normal man, the pulsatile fraction of renin release exactly reflects the sleep stage pattern, with increased renin release when sleep becomes deeper, and reduced release when sleep becomes lighter. These studies were carried out to determine whether this relationship persists in the case of sleep disorders such as narcolepsy and sleep apnea, and in case of renin disorders, such as low renin essential hypertension.

Narcolepsy is characterized by sleep attacks in the day-time, frequent awakenings during nocturnal sleep and sleep onset REM episodes (SOREM). Renin profiles reflected all these irregularities in the sleep structure and the normal sleep-related periodicity of renin release did not occur.

Restoration of regular NREM-REM sleep cycles by treatment with nasal continuous positive airways pressure in sleep apnea patients restored the normal renin oscillations, which had disappeared due to marked sleep fragmentation in untreated patients. This led to enhanced aldosterone release, which counteracted the high water and salt losses occurring in untreated sleep apnea patients.

In low renin essential hypertension, the increases associated with NREM sleep were small, but the mean relative amplitude expressed as a percentage of the nocturnal mean was about 60 %, which was similar to that in normotensive subjects. Following single or repeated doses of an angiotensin-converting enzyme inhibitor, the oscillations were greatly amplified, which revealed that their relationship to specific sleep stages was not disturbed.

The results give evidence of the strength of the sleep-related processes generating the oscillations, which invariably remained linked to the NREM-REM sleep cycles. In none of the pathological cases was this association disrupted.

42 THE TIMING OF CARDIAC ARRESTS

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Reports on the incidence of cardiac death in the community[1 2 3] have described a peak in the morning hours (7-11h). A bimodal distribution has also been suggested, with a secondary peak in the evening[1]. Do these variations represent a true rhythm of cardiac arrests or reflect merely a rhythm in the reporting of such events?

Purpose To investigate the timing (over 24 hours) of out-of-hospital cardiac arrests in Ontario.

Methods Cardiac arrest victims taken to hospital by ambulance in five Ontario communities over a two-year period were included. Time of arrest was obtained from ambulance, emergency and hospital records.

Results 1080 cases were examined. Plotting the incidence of arrest over time revealed a trough in the early morning hours, and two peaks: 08-11h and 19-20h. Fewer arrests occurred in the block 00-06h compared to 06-12h, 12-18h and 18-24h (χ^2= 53.8, df=3, p<.005). A two-harmonic fit was statistically significant [F(4,19) = 15.8, p<.0001].

Conclusions Because this study had the combined advantages of a large sample size and carefully researched time-of-arrest estimates, the described bimodal distribution is less likely due to a rhythm in the reporting of arrests. Hence it appears that the chances of cardiac arrest are greatest mid-morning and mid-evening. This has implications for theories of the etiology of sudden cardiac arrest and for the scheduling of emergency health personnel.

[1]Muller JE et al. Circulation 1987;75(1):131-8. [2]Willich SN et al. Am J Cardiol 1987;60:801-6. [3]Mitler MM et al. Am J Med 1987;82:266-74.

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43 EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON EGG LAYING IN A MARINE MOLLUSC, *APLYSIA CALIFORNICA*. Nancy L. Wayne and Gene D. Block, Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

The marine mollusc *Aplysia californica* is a seasonal breeder, laying eggs primarily in the late summer-autumn and reproductively quiescent during the winter and spring. The purpose of this study was to investigate the effects of photoperiod and water temperature on the frequency of egg laying. Animals were collected from California at 4 different times of year, and upon arrival in the laboratory they were housed in temperature- and light-controlled aquaria. Animals were kept in individual, perforated buckets within the aquaria and monitored for egg masses daily for 3-5 weeks. Animals that arrived in early-AUTUMN were divided into 4 groups: short days (8 hr light/day; 8L) and warm water (20°C); short days and cold water (8L; 15°C); long days and warm water (16L; 20°C); long days and cold water (16L; 15°C). Animals in warm water layed eggs more frequently than those in cold water ($p<0.01$). Furthermore, animals kept in short days and warm water layed eggs more frequently than those in long days and warm water ($p<0.01$). There was no difference in egg laying between the 2 cold-water groups (animals in cold water showed little to no egg laying). Animals that arrived in both early-WINTER and early-SPRING were kept in either short days and warm water (8L; 20°C) or long days and warm water (16L; 20°C). At these 2 times of year, animals showed little or no spontaneous egg laying throughout the study (and no photoperiodic response was detected). Importantly, all animals could be hormonally induced to lay eggs at the end of each study, indicating that they were reproductively mature. Animals that arrived in early-SUMMER were kept in either short days and warm water (8L; 20°C) or long days and warm water (16L; 20°C). As with the AUTUMN animals, SUMMER animals kept in short days layed eggs more frequently than those kept in long days ($p<0.05$). Overall, these results suggest that warm water is permissive for egg laying and that short days can further stimulate this behavior. However, there is a strong inhibition of spontaneous egg laying during the winter and spring, which neither warm water nor short photoperiod can overcome. We are currently studying the electrophysiological properties of the reproductive neuroendocrine cells controlling egg laying under different photoperiodic and water-temperature conditions. (Supported by NIH-NS-08725 to NLW and NIH-NS-15264 to GDB).

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NOVEL COMBINATIONS OF CIRCADIAN ECLOSION RHYTHM AND PHOTOPERIODIC DIAPAUSE IN LABORATORY STRAINS OF DROSOPHILA LITTORALIS

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Drosophila littoralis is distributed in Europe from northernmost Scandinavia (70 °N) to the Mediterranean region and the Caucasus mountains (40 °N). The time measuring system is genetically highly variable and latitude dependent. The critical daylength for photoperiodic adult diapause varies from 12 h in the south to 20 h in the north. The phases of the pupal eclosion rhythm in entraining light/dark -cycles vary correspondingly, being 9 hours earlier in the north than in the south. The amplitude of the free-running rhythm is lower, and the rhythm has earlier damping in the north.

In earlier studies, crossing and selection experiments were made and observed for up to sixteen generations. In those experiments, parallel changes in diapause and eclosion traits were observed. It was concluded that at least a part of the variation of the traits was caused by common genes.

In the present study, the above hybrid populations were re-examined after 30 - 50 generations of free recombination. In many cases, a combination of extremely southern diapause and northern eclosion characteristics was found in a single strain. These combinations have never been found in natural populations, and are now seen for the first time in "synthetic" strains as well. Conclusively, the genes controlling the differences in circadian rhythmicity and photoperiodism between strains of D. littoralis are different but very closely linked.

45 **RESPONSIVENESS OF THE HAMSTER REPRODUCTIVE APPARATUS TO A SINGLE LONG DAY: IS THERE MAXIMAL SENSITIVITY AT WEANING?** Cynthia M. Finley and Carol S. Whaling. Department of Psychology, University of California, Berkeley, CA 94720

Exposure to a single long day stimulates gonadal growth over the course of the next two weeks in weanling Siberian hamsters (Spears et al, 1990). The present experiment sought to determine whether the immediate postweaning interval constitutes a critical or sensitive period during which hamsters are uniquely responsive to light. Male hamsters were housed from birth to weaning (day 18) in a long photoperiod (16 h light/day) and thereafter in a short photoperiod (8 h light/day). At 50 or 70 days of age groups of males received either an additional short day (control groups) or one extended long day (experimental groups). All groups were housed in the short photoperiod for 17 days after these treatments; autopsies were performed at 67 and 87 days of age, respectively. Testes weights of experimental vs control groups were 122 ± 49 vs 108 ± 51 mg at 67 days, and 338 ± 132 vs 266 ± 107 mg at 87 days of age. The single long day did not stimulate testes growth at 50 and 70 days of age. Similar treatment at 18 days of age, however, produced highly significant increases in testes weight. We conclude that sensitivity to photoperiod is greatest at weaning.

46 PHOTOPERIODIC INFLUENCES ON BEHAVIOR REQUIRE THE PINEAL IN MALE AND FEMALE SYRIAN HAMSTERS. Jonathan Karp, Michael Miernicki, and J. Bradley Powers, Department of Psychology, Vanderbilt University, Nashville, TN 37240.

The role of the pineal gland in mediating short photoperiod-induced changes in testosterone (T) and estrogen (E) sensitive behaviors was assessed in male and female hamsters (*Mesocricetus auratus*). In one experiment, males (n=36) were group-housed in a long (LP; 14L:10D) or short (SP; 8L:16D) photoperiod. After 2 weeks, all males were castrated; hamsters in LP were pinealectomized (LP/PX) and those in SP were either pinealectomized (SP/PX) or given a sham-operation (SP/SH-PX). Copulatory behavior (CB) impairments following castration developed more rapidly in the SP/SH-PX, compared to either the SP/PX or LP/PX groups, over the next 8 weeks. CB tests during weeks 6 and 8 indicated that only 8% of SP/SH-PX hamsters mounted, but 65% or more of the LP/PX and SP/PX males exhibited this behavior. Hamsters were then given T-filled Silastic capsules sc. This treatment restored CB within 2 weeks in LP/PX and SP/PX males, but behavioral restoration was incomplete among SP/SH-PX males. Thus SP conditions impaired the expression of CB, both in the absence and presence of T, but only in males with an intact pineal gland.

In a second experiment, females (n=32) were divided into 3 treatment conditions based on average daily wheel running activity measured over 2 weeks in LP (16L:8D). Activity wheels were then removed and 2 groups were switched to SP, 1 remained in LP; appropriate pineal surgery produced LP/PX, SP/PX, and SP/SH-PX groups. After 11 weeks, females were ovariectomized; E-filled or empty Silastic capsules were implanted sc. Daily activity was again measured for 3 weeks, and during this period lordosis was tested on 3 occasions in 10 minute tests with males. Activity levels were significantly increased by E among the 2 pinealectomized groups. Average daily wheel revolutions (x 1000) were 9.2 ± 1.0 vs. 5.2 ± 1.3, and 7.6 ± 1.4 vs. 3.2 ± 1.2 for E vs. empty implants in the LP/PX and SP/PX groups, respectively. This effect of E was absent in the SP/SH-PX females (3.6 ± 1.2 vs. 4.3 ± 1.1). Lordosis was facilitated by E in approximately the same proportion of females among the 3 groups (LP/PX - 71%; SP/PX - 75%; SP/SH-PX - 67%); females with empty implants were unresponsive. E was then removed to allow its priming effects to dissipate. After 7 days, progesterone (250 ug) was injected; this facilitated lordosis in the 50% or more of the LP/PX and SP/PX groups, but in 0% of the SP/SH-PX females. Our results support the hypothesis that photoperiodic effects on behaviors regulated by gonadal steroids in both male and female hamsters require the pineal gland for their expression. The role of melatonin in transducing these photoperiodic effects is now being investigated.

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47 EFFECT OF A SIMULATED NATURAL PHOTOPERIOD CYCLE ON TIMING OF ANNUAL CYCLES IN TWO HAMSTER SPECIES, Jeffrey A. Elliott and Bruce D. Goldman, Department of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269-4154

We wished to determine whether a simulated natural photoperiod cycle (NPP) would influence the timing of annual cycles displayed by Siberian and Turkish hamsters. Adult male and female hamsters raised in 16L:8D were exposed to one of 3 photoperiod treatments: 8L - constant 8L:16D for 48 weeks; NPP - NPP cycle (50 degrees N. latitude) for 48 weeks; NPP/8L - NPP cycle as above for weeks 0-22, followed by transfer to 8L for weeks 23-48. Daylength in NPP began at 16h, reached a nadir (L=8h 5min) at week 23 and increased thereafter.

In male Turkish hamsters, 8L induced testicular regression and concurrent declines in serum prolactin (PRL) and testosterone to basal levels after 8-12 weeks, followed by a return to "long-day" values by week 32. A similar pattern of regression and recrudescence (RR) occurred in NPP and NPP/8L, except that the RR curve was phase-delayed 4 weeks compared to 8L. Exposure to 8L during weeks 23-48 did not alter the timing of recrudescence. NPP and NPP/8L females resumed regular estrous cycles approximately 4 weeks later than did 8L females.

In male Siberian hamsters, regression of body weight and testis width occurred later in NPP and NPP/8L as compared to 8L, but testicular recrudescence was not phase-delayed. Recrudescence of body weight was delayed only in the NPP group; this delay was associated with elevated serum PRL at weeks 36-48. We found no compelling evidence for differential effects of the 3 photoperiod treatments on the pelage color cycle in either sex or on body weight and PRL in females.

Our results suggest that the primary influence of NPP is to cause a delay in the regression phase of the annual cycle. With the exception of the above mentioned effects on male Siberian hamsters, they do not support the hypothesis that a gradual increase in daylength is important in regulating the timing of recrudescence in Turkish or Siberian hamsters.

48 PROLACTIN-DEPENDENT SEASONAL CHANGES IN PELAGE: ROLE OF THE PINEAL GLAND AND DOPAMINE. Lori L. Badura & Bruce D. Goldman, Dept. Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269.

In the Siberian hamster (Phodopus sungorus sungorus), seasonal changes in pelage color are dependent upon an interaction of photoperiod-mediated fluctuations in basal prolactin (PRL) and gonadal steroid levels. Exposure to a short-day photoperiod for several weeks results in decreases in both basal PRL and circulating steroid levels, and the animals subsequently molt to the characteristic white winter pelage. Chronic administration of exogenous PRL prevents the short-day induced molt. The role of the pineal gland and the dopaminergic system in the mediation of this seasonal event was assessed via both photoperiodic and pharmacological manipulations of basal PRL levels.

All animals were castrated, received either pinealectomy (PINX) or sham-pinealectomy procedures, and were maintained under either a long (16L) or short (10L) photoperiod. Basal PRL levels are largely under the control of the inhibitory effects of dopamine (DA). The animals thus received one of three pharmacological conditions in order to artificially manipulate circulating PRL: 1) a DA agonist, bromocryptine (CB154; 200 µg/ml) for the first 8 weeks, 2) a DA antagonist, pimozide (20 µg/ml) for weeks 5-17, or 3) vehicle (control) throughout. All agents were dispensed orally via the drinking water. Body weight, pelage condition, and PRL levels were assessed at various intervals during a 7.5 month period. Regardless of pharmacological condition, none of the sham or PINX animals in 16L showed any evidence of pelage changes. Likewise, none of the PINX animals in 10L underwent the winter molt. In contrast, sham animals in 10L receiving control solution showed clear evidence of pelage change after 8 weeks of exposure to short days. Administration of CB154 accelerated the molt, while pimozide prevented the molt until its removal at week 17. These results indicate that while basal PRL levels under different photoperiodic conditions are partly regulated by the actions of the DA system, the pineal-dependent inhibition of PRL in short days cannot be entirely explained by an increase in DA activity.

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LIGHT-MEDIATED GONADAL GROWTH INDEPENDENT OF SUSTAINED PINEAL SECRETORY ACTIVITY IN SIBERIAN HAMSTERS. Carol S. Whaling[1] and Cynthia M. Finley[2], [1]Group in Endocrinology and [2]Department of Psychology, University of California, Berkeley, CA 92720.

Seasonal reproduction in the Siberian hamster is controlled by photic cues. Testes undergo regression, uteri involute and body weight is reduced in short day lengths. We have demonstrated previously that a single day of extended light at weaning is sufficient to stimulate gonadal growth in animals maintained in short day lengths (Spears et al, in press). These results suggest that acute light stimuli have prolonged effects on one or more of the neural or hormonal components involved in the transmission of photoperiodic information. The pineal gland is essential for photoperiodic response in Siberian hamsters (Hoffmann, 1973). In this study we tested temporal parameters of pineal action by removing the pineal gland 72 hours after the light treatment.

Hamsters were raised in a 8L:16D photoperiod to 19 days of age and were assigned to one of three groups. The first group was given extended light on day 19 (33 hours of continuous light) and pinealectomized on day 22. The second group was given the same light treatment but sham-operated on day 22. The third group was not given the light treatment and was pinealectomized on day 22. All animals remained in 8L:16D until autopsy at 35 days of age. Light-treated, pinealectomized hamsters had significantly heavier gonads than pinealectomized hamsters that did not receive light treatment. We conclude that light stimulated gonadal growth can occur in the absence of pineal secretory activity 72 hours after the light treatment. Altered melatonin secretion initiated by the single light treatment presumably has prolonged effects on mechanisms governing gonadotropin secretion.

50 EFFECTS OF NEAR-ULTRAVIOLET RADIATION ON MELATONIN RHYTHMS AND REPRODUCTIVE DEVELOPMENT IN SIBERIAN HAMSTERS. Karen T. Stewart, John P. Hanifin, Mark D. Rollag, Milton Stetson, and George C. Brainard. Dept. of Neurology, Jefferson Medical College, Dept. of Anatomy, Uniformed Services University of Health Sciences, and School of Life and Health Sciences, University of Delaware.

It has recently been shown that near-ultraviolet radiation (UV-A), though thought to be outside the range of visual perception, can affect circadian, neuroendocrine, and reproductive responses in some rodent species. Here we present two studies which suggest that schedules of UV-A exposure can influence melatonin rhythms and gonadal development in Siberian hamsters (*Phodopus sungorus*).

The first study investigated the effect of long or short day cycles of UV-A on the pineal melatonin rhythm. Adult female hamsters (n=131) were exposed to broadband UV-A either from 0900-1700 (LD 8:16) or from 0100 to 1700 (LD 16:8) for 19 days. Groups of animals from each lighting condition were then sacrificed in darkness at 0100, 0300, 0600, 0900, 1100, 1400, 1700, 1900, 2100, and 2300. Pineals were removed and assayed for melatonin content using RIA. Data were analyzed using 2-way ANOVA, and comparisons were made of treatment cell means at selected times of day, using t-tests with Bonferroni corrections of significance levels. There were significant main effects of both photoperiod ($F=15.84$, $df=1$, $p<.0001$) and time of day ($F=11.55$, $df=9$, $p<.0001$), and a significant interaction ($F=2.635$, $df=9$, $p<.001$). Melatonin levels in both groups began to rise at 1900 and reached peak levels at 2300. Melatonin production had ceased by 0300 in the long photoperiod group, but was still elevated at 0300 in the short photoperiod group ($t=5.97$, $p<.0001$), and at 0600 in some individual short photoperiod subjects. Therefore, the duration of elevated melatonin production lasted 3-6 hours longer in animals housed in short UV-A days. Melatonin was low during the hours of UV-A exposure in both groups. Thus, the rhythm of pineal melatonin production in Siberian hamsters appears to respond to photoperiods of UV-A light in a manner similar to photoperiods of visible white light.

In a second study, juvenile male hamsters (n=27) were housed for 17 days in either long days (LD 16:8) or short days (10:14) of white light. A third group was housed in short days (LD 10:14) with a 15-minute pulse of monochromatic UV-A radiation (340 nm) 5 hours after lights-out. After sacrifice, testes weights were analyzed using ANOVA and the Newman-Keuls test. There was a significant treatment effect ($F=20.15$, $df=2$, $p<.01$), with short day subjects exhibiting significantly smaller testes than the other groups ($p<.001$). The long day subjects and the UV-A subjects did not differ from each other. Thus, a daily pulse of UV-A radiation blocked the effects of short photoperiod on testicular development. These studies indicate that UV-A exposure can regulate reproductive development and melatonin rhythms in this species. Supported by NEMA (LRI89:DR:NEMA:1); NASA (NAGW1196); NSF (DCB84-12587 and DCB87-14638); and USUHS (CO7049).

51 SHORT-DAY INDUCED REGRESSION IN MALE MEADOW VOLES MAY BE REVERSED BY BRIEF EXPOSURE TO A LONG PHOTOPERIOD. Leslie R. Meek, Gretchen D. Reeves, Theresa M. Lee & John Dark. University of Michigan, Ann Arbor, MI & University of California, Berkley, CA.

Spears,et al (1990) reported that prepubertal Djungarian hamsters maintained from birth in a short day length (SD) undergo pubertal development at the same age as pups housed in a long daylength (LD), if exposed to as little as 15 minutes of extra light at 18 days of age. We were interested in 2 questions: 1) would another species, the meadow vole (Microtus pennsylvanicus) display a similar juvenile phenomenon; 2) would regressed adults respond to a short period of extra light by undergoing recrudescence?

Experiment 1. Male meadow voles reared from birth to 50 days of age in SD were exposed to 2 days of constant light (SD+LL) and thereafter remained in SD. At 70 days of age the SD+LL pups had equivalent body weight, paired testes weight and brain weight to (LD) controls, and were significantly heavier than SD controls. Thus, juvenile meadow voles undergo rapid pubertal development after 2 days of extended light, despite returning to short daylengths.

Experiment 2. Adult male meadow voles were maintained in SD for 8 weeks prior to exposure to varying periods of extra light. Photoperiod manipulations at 8 weeks were: 8 wks of SD followed by 2 long days (8+2LD); 8 wks of SD followed by 1 long day (8+1LD); 8 weeks of SD with 2 extra hr of light (8+2hr). Experimental animals returned to SD for 4 weeks after light treatment and then were autopsied. Pelage changes indicative of a molt were found in all SD animals exposed to extra light; hair depth and density were reduced as compared to SD controls and guard hairs were longer as they more easily released from the follicle. Additionally, SD+2LD animals had lower epidydimal fat weights as compared to all other groups. These data indicate that the extra light treatment triggered the spring molt.

Adult animals do not respond as rapidly as prepubertal juveniles to a brief increase in day length, perhaps indicating a basic difference between pubertal responses to day length and adult recrudescence. Data from histological analysis and additional experiments in progress, comparing juvenile and adult sensitivity to extra light exposure will be presented at the meeting.

52 ENDOGENOUS CIRCANNUAL RHYTHMS OF REPRODUCTION IN EWES: EFFECTS OF EXPOSURE TO CONSTANT PHOTOPERIOD AND PINEALECTOMY. D. O'Callaghan, *F.J. Karsch, M.P. Boland and J.F. Roche, University College Dublin, Ireland and *Reproductive Sciences Program, The University of Michigan, Ann Arbor, MI. 48109.

Endogenous circannual rhythms exist in many species, including sheep. Our objective was to determine if circannual rhythms of reproduction persist in ewes under constant photoperiod or in the absence of the pineal. Ovariectomized ewes treated with estradiol were given 1) long days (LD, 17L:7D, n=8), 2) short days (SD, 8.5L:15.5D, n=6), or 3) pinealectomized and kept on short days (PX, n=14). Reproductive activity was assessed by measuring LH in blood samples every 2 weeks; high LH indicates reproductive induction and low LH, reproductive inhibition. Cycles of LH were analyzed using a stepwise algorithm which identified sequential stages of high and low LH. The median duration of high and low LH, cycle period (rise to rise) and amplitude of LH cycles were determined.

Results are reported for the first 3 years of the study. Among the 8 ewes exposed to LD, 5 had repeated cycles, 2 remained reproductively inactive and 1 had a single cycle. Of the 6 ewes exposed to SD, 4 had repeated cycles, 1 had a single cycle and 1 was reproductively inactive. Of the 14 PX ewes, 6 had repeated cycles, 6 had a single cycle and 2 were reproductively inactive. The median duration of reproductive induction was not different between treatments (LD=104±13, SD=93±14, PX=91±20 days (±sem), $p>0.05$). The duration of reproductive inhibition was shorter in ewes exposed to LD (180±15 days) compared to SD, (300±45 days, $p<0.05$); the duration of reproductive inhibition in PX ewes (260±44 days) was not different from the duration in ewes on LD or SD. The period of the complete reproductive cycles identified was not different between treatments (LD=256±14, SD=334±50, PX=275±28 days, $p>0.05$). The amplitude of the LH cycle was greater in ewes on LD (3.8±1 ng/ml) or SD (6.2±2 ng/ml) than in PX ewes (1.7±0.2 ng/ml, $p<0.001$); amplitude of LH in ewes on LD and SD was not different. These results show that endogenous circannual rhythms of reproduction are expressed in the majority of ewes under constant long or short days; certain characteristics of the rhythm may depend upon the specific photoperiodic environment. Pinealectomized ewes also expressed endogenous rhythms, indicating that the pineal is not required for the expression of the rhythm, although it may be important in determining the amplitude of the cycle. (UCD and NSF INT-8608943).

53 **ABOLITION OF THE SEASONAL VARIATIONS OF SEXUAL ACTIVITY IN THE HE-GOATS BY A RAPID ALTERNATION BETWEEN LONG AND SHORT DAYS.**
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He-goats are highly seasonal breeders. The sexual activity is high in autumn and winter and low in spring and summer. This is controlled by photoperiod. Two groups (n=6) of one-year-old French Alpine and Saanen he-goats were used to determine if seasonal sexual variations can be abolished using a rapid alternation of long and short days. The Experimental group (E) was housed in a light proof building for 3 years. The animals were subjected to an alternation between one month of long days (16L:8D) and one month of short days (8L:16D). The Control group (C) was kept in open sheds under natural daylength (extremes: 16L:8D and 8L:16D). Testicular weight was measured twice a month by comparative palpation. Plasma concentration of testosterone was determined from a weekly blood sample and sperm production was assessed twice a week. The results of sperm production are presented only for the first two years.

Testicular weight of males from Group C showed large seasonal variations (first year from 72 ± 3 g, mean ± sem, in May to 127 ± 4 g in September), whereas, in Group E, these seasonal variations were attenuated (95 ± 8 g and 114 ± 4 g, respectively). During the second and third years, seasonal variations persisted in Group C: 109 ± 8 g in January and 149 ± 1 g in October but, were abolished in group E: 140 ± 2 g and 153 ± 1 g, respectively. Similar seasonal variations of mean testosterone levels are also observed in Group C: minimal and maximal testosterone levels were 1,3 ± 0,2 ng/ml in February and 17,1 ± 1,5 ng/ml in September. In contrast, in Group E, testosterone presented variations which were synchronized by daylength: 1,3 ± 0,8 ng/ml in short days and 10,6 ± 1,8 ng/ml in long days. Sperm production during the first year, presented large variations in both groups, Group C: from 2,8 ± 0,3 in March to 4,6± 0,6 x 10^9 spz/ejaculate in October; Group E: respectively 2,6 ± 0,5 and 6,7 ± 0,9 x10^9 spz/ejaculate. During the second year, seasonal variations persisted in Group C, whereas in bucks from Group E, sperm production remained constantly high. The total number of sperm produced per ejaculate in the second year is larger in Group E (6,3 ± 0,3 x 10^9) than in Group C (4,2 ± 0,4 x 10^9).

These data demonstrate that the rapid alternation between long and short days abolished seasonal variations of the gametogenetic activity (testicular weight and semen production) and reduced the testosterone variations observed normally in the French Alpine and Saanen he-goats.

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SOCIAL ENTRAINMENT IN TREE SHREWS M. Menaker, M.A. Vogelbaum and N. Kassell, Division of Biomedical Engineering and Department of Biology, University of Virginia, Charlottesville, Virginia 22901.

The effect of social interaction on the freerunning period and phase of the locomotor rhythm of tree shrews (Tupaia belangerie) was investigated. Tree shrews were maintained in constant light, the intensity of which was sufficient to prevent splitting of the locomotor rhythm, in separate cages, two cages to a room. The cages were one to two feet apart and each was equipped with a running wheel. Visual contact could be blocked with a physical barrier, but auditory and olfactory cues were always unimpeded.

In one case two males were kept in one room for 3 months. During this time the rhythm of one male freeran with a period that averaged about 23.1 hours. There did not appear to be any consistent influence of the activity of the other tree shrew on the period of its rhythm. The other male showed a consistent acceleration of its rhythm, from about 23.8 hours to 23.1 hours, over the course of the experiment. The greatest change in period occurred when the phases of the two rhythms overlapped. The animals have not yet been separated to remove the social stimuli.

A male-female pair were maintained in another room for 2 months. Throughout this experiment, the female ran with a period of about 23 hours. The male started with a period of close to 24 hours, and after 2 months the period was close to 23 hours. At this point, the male's locomotor activity was 180^o out of phase the female's; the male ran while the female was inactive and vice-versa.

Tupaia are known to have a complex social structure in which males form dominate-subdominant-submissive relationships, and males and females may pair bond. Social relationships can dramatically affect the physiological state of individual tree shrews. The preliminary results reported here suggest that Tupaia may be a useful model for investigating the effects of social interactions on the circadian system.

55 **WHEEL RUNNING ACTIVITY IS A DETERMINANT OF CIRCADIAN RHYTHM PERIOD IN THE MOUSE.** Connie E. Martin, Dale M. Edgar and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94304.

It has long been known that the period of free-running circadian rhythms can be influenced by specific environmental factors (e.g., light intensity). Cage environments, and some drugs have also been shown to influence rhythm period. With recent experiments suggesting that vigorous activity may phase-shift the biological clock, it is plausable that the "manifest" circadian rhythm period may be dependent on the level and timing of spontaneous activity behaviors. This study examines this possibility by comparing the free-running period of sleep-wake and drinking circadian rhythms as a function of running wheel availability.

Eight male mice (Mus musculus, C57BL/6NNia, 6-12 mos) were surgically prepared for continuous EEG and EMG recording. Each cage contained a commutator which allowed the animals freedom of movement and a beveled running wheel. Sleep-wake, drink and running wheel activity were monitored using SCORE, a microcomputer based sleep scoring and data collection system. Mice were maintained in constant darkness with wheels free to rotate for approximately 13 weeks. Once stable free-running circadian rhythms were established, the wheels were mechanically locked (19 weeks), after which the wheels were again released and free to rotate (8 weeks). Mice were housed individually in sound-attenuated chambers with food and water available ad libitum, and an ambient temperature of 23-24C. Mean circadian rhythm period was computed for each animal using a minimum variance periodogram technique applied to 20 day segments of the sleep-wake data.

With running wheels available mice exhibited robust sleep-wake and drinking circadian patterns with a period of 23.43±0.08. Spontaneous running activity was concentrated during the first 3-4 hours of the subjective night (CT-12 to CT-16), and drinking and consolidated bouts of wakefulness were also most prevalent at this time. When running wheels were locked, marked attenuation of sleep-wake circadian rhythms was observed along with a significant increase in mean period (tau = 23.89±0.04, $p < 0.03$ Paired t-Test). When the running wheels were again free to rotate, the circadian rhythm period decreased (tau = 23.61±0.04, $p < 0.01$) and a robust circadian waveform was reestablished.

These findings show that the "manifest" endogenous rhythm period is determined, in part, by spontaneous behavioral activities (e.g., wheel running exercise). These data are also consistent with the hypothesis that spontaneous exercise/activity provides behavioral feedback to the biological clock, and that vigorous activity occuring early in the waking half of the circadian cycle imposes small phase advances on the circadian timekeeping system.

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56 IS THE PHASE-SHIFTING EFFECT OF TRIAZOLAM ON THE HAMSTER'S CIRCADIAN CLOCK MEDIATED BY A CHANGE IN BODY TEMPERATURE? Carmen Wickland, Fred W. Turek. Dept of Neurobiology & Physiology, Northwestern University, Evanston, Illinois 60208

Treatment with the short-acting benzodiazepine, triazolam (Tz), given six hours before activity onset (CT 6), induces large phase advances in the circadian rhythm of locomotor activity of golden hamsters and also induces an acute increase in locomotor activity. Restraining an animal for a six hour period of time beginning immediately after injection of Tz at CT 6 will block the phase shift normally induced at this time, while restraint alone during this time induces no phase shift in the activity rhythm. Since increased locomotor activity is associated with a rise in body temperature in small rodents, this study was designed to determine whether the phase-shifting effect of Tz on the circadian clock could be mediated by the change in body temperature associated with the induced increase in locomotor activity.

Twelve animals were implanted with Mini-Mitter telemetry devices and housed in constant light in cages equipped with running wheels for 3 weeks prior to the first experiment. The animals were then either (1) injected with 2.5 mg Tz at CT 6 (Tz), (2) injected with Tz at CT 6 and restrained for 6 hours (Tz+r), or (3) restrained for 6 hours beginning at CT 6 (r). At intervals of at least two weeks, the animals received a different treatment. Body temperature change was measured as the difference between the 10 min. values for 6 hrs. after CT 6 on the treatment day and the means of the corresponding circadian time points on five control days.

Mean body temperature for each 10 min. interval increased over control temperatures for all three groups during most of the 6 hours following the beginning of the treatment (Tz, n=9; Tz+r, n=8; r, n=9). There was no significant difference between any pairs of groups for the 6 hours. The phase shifts in the total activity rhythm induced by Tz, Tz+r, and r, were +100.0±13.0 min. (n=7), -16.4±7.4 min. (n=7), and -2.5 ±11.5 min. (n=8) respectively.

These results indicate that the phase shifts in the circadian rhythm of activity induced by Tz are not mediated by the resulting increase in body temperature, since (1) restraint alone induces an increase in body temperature but does not induce phase shifts in the activity rhythm, and (2) restraint blocks Tz induced phase shifts but not the increases in body temperature.

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RESTRAINT DOES NOT BLOCK THE PHASE SHIFTING EFFECTS OF A PROTEIN SYNTHESIS INHIBITOR ON THE HAMSTER CIRCADIAN CLOCK. Deborah A. Hinch and Fred W. Turek. Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Systemic administration of the protein synthesis inhibitor, cycloheximide (CHX), is capable of inducing both phase advances and delays in the circadian rhythm of locomotor activity of hamsters (Mesocricetus auratus) maintained in constant light. The phase response curve to CHX is similar to those for pulses of darkness on a background of constant light or for administration of the short-acting benzodiazepine, triazolam. The phase shifting effects of dark pulses or triazolam can be totally suppressed by immobilization of the hamsters during treatment. This indicates that these effects are mediated through a change in the activity state of the animal. In order to determine whether or not the effect of CHX on the clock is also mediated through a change in the activity state of the animal, the effects of immobilization were tested on the phase shifting properties of CHX. Phase shifts in the rhythm of locomotor activity of animals given either subcutaneous CHX injections, 6 hours of restraint (R) or both treatments were determined. The treatments were given at either the circadian time in which CHX produces the maximum phase advance (CT 6) or the maximum phase delay (CT 0). Restraint did not block the characteristic phase advances and delays induced by CHX administration at either CT 6 (CHX: +34 $\pm$ 9 min; CHX+R: +38 $\pm$ 10 min) or CT 0 (CHX: -64 $\pm$ 7 min; CHX+R: -66 $\pm$ 10 min). Restraint alone had no signifigant effect on the locomotor activity rhythm of the animals at the circadian times tested (CT0: 0 $\pm$ 4 min advance; CT6: 2 $\pm$ 6 min delay). The similarities between phase shifts observed in response to cycloheximide and phase shifts observed in response to triazolam or dark pulses, but the different response to immobilization, suggest that these stimuli initially follow different pathways to the biological clock, although these stimuli may ultimately converge to act on the circadian clock via similar mechanisms.

58 CHRONIC EXERCISE HAS NO EFFECT ON SERUM MELATONIN LEVELS IN FEMALE HAMSTERS ON LONG OR SHORT PHOTOPERIOD. David R. Pieper, Catherine A. Lobocki and Katarina T. Borer
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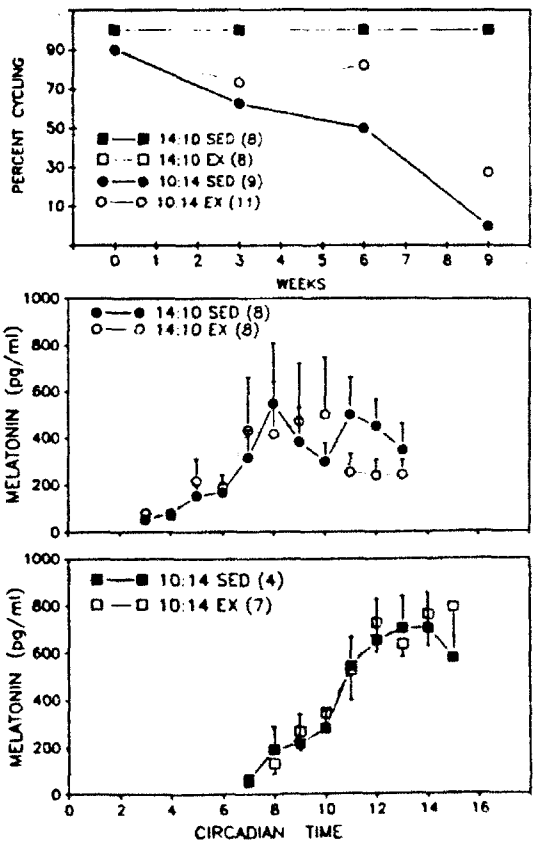
When hamsters are given access to a running wheel, they voluntarily run between 5 and 10 km per night. This exercise tends to inhibit the cessation of estrous cycles which accompanies maintenance of the animals on short days. The experiment reported here tested the hypothesis that exercise influences the response to photoperiod by altering the nocturnal elevation in melatonin secretion.

Thirty-six female Syrian hamsters were divided into 4 groups. One group was placed in cages without running wheels (SED) on long days (14L:10D). A second group was placed in cages with running wheels (EX) in long photoperiod, and there was also a SED and EX group on short days (10L:14D). Twelve weeks later, the animals were fitted with chronic catheters, and were bled hourly during the time of the nocturnal rise in melatonin.

All hamsters on long photoperiod had regular estrous cycles throughout the study. All 9 hamsters in the SED group on short photoperiod became anestrus but 7 of 11 EX animals on short days continued to have regular estrous cycles. There was no difference in melatonin concentrations between the EX and SED groups on either photoperiod, and there was no difference in the melatonin levels of EX hamsters on 10L:14D that became anestrus versus animals in the same group that continued to cycle.

In conclusion, the interaction between exercise and response to photoperiod is not due to an alteration in melatonin secretion in female hamsters.



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59 **THE ROLE OF AMINERGIC AGENTS IN CIRCADIAN TIMEKEEPING.** Peter P. Sayeski, Dale M. Edgar, Joseph D. Miller, and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94305.

The free running rhythm of the Golden Hamster can be phase shifted by various means (light, melatonin, benzodiazepines). Recently, activity has been implicated in producing phase shifts as well. However, the mechanisms responsible for activity-related phase shifts are unknown. Since the levels of central monoamines are known to change markedly with activity and arousal state, we investigated whether free-running circadian rhythms in hamsters could be altered by manipulating monoaminergic transmission using d-amphetamine and serotonegic agents.

Male Golden Hamsters (Mesocricetus auratus, 3-4 mos.), were used in this study. Animals were housed in Nalgene "F" size cages equipped with running wheels and maintained in constant darkness (DD). Food and water were available ad libitum. All animals were allowed at least 17 days between drug treatments. The animals received a single dose of d-amphetamine (1mg/kg, IP) at one of six different circadian times (CT 0, 6, 9, 12, 18, or 21). This dose is believed to be sufficient to increase levels of norepinephrine and dopamine in the CNS and to induce behavioral hyperactivity. However, no significant phase shifts (> 10 min) were observed, nor were there any changes in tau following treatment. The animals then received a single dose of fluoxetine (10mg/kg, IP) at either CT-6 or CT-18. This dose significantly increases 5HT levels in the CNS by selectively inhibiting its re-uptake and was found to induce an 8 to 10 hour state of quiescence. Roughly half of the animals (4 of 8 at CT-6 and 4 of 7 at CT-18) exhibited transitory waveform disruptions lasting only two to three days (corresponding to the half-life of the drug). However, there was no evidence of drug-induced phase shifts. The animals subsequently received a single dose of CGS12066B (0.1mg/kg, IP) a 5HT-1b presynaptic agonist, administered at either CT 6 or CT 18. Again, some animals showed transitory changes in waveform, but no significant phase shifts. Finally, these animals were injected with a single dose of melatonin (0.01mg/kg, SC) at either CT-10 or CT-22. In contrast to the animals treated with the other monoaminergic agents, the CT-10 melatonin group exhibited a 30-min phase advance on average compared to control injections, while some, though not all, of the CT-22 animals exhibited small (< 20 min) phase delays.

These results suggest that enhanced release of the classical monoamines, or the direct activation of the 5HT-1b receptor does not influence the circadian clock. In contrast, the indoleamine melatonin did generate phase shifts. The mediating mechanism for triazolam or dark pulse phase shifts appears to involve locomotor hyperactivity. Interestingly, in this study amphetamine induced hyperactivity was not associated with phase shifts. That this dose of amphetamine actually seemed to impair coordinated locomotor function, although the animals were hyperactive by other measures, suggests that stimulants may only be effective in producing phase shifts if they raise activity levels without impairing integrated motor sequences.

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60 DIETARY INDUCED OBESITY INFLUENCES ANTICIPATORY ACTIVITY IN RATS. Judith E. Persons and Friedrich K. Stephan, Department of Psychology, Florida State University, Tallahassee, Florida.

Increased wheel running in anticipation of food access has been demonstrated in rats under conditions of restricted feeding (RF). In this study, the influence of body weight differences induced by diet on anticipatory activity(AA) was examined.

Twenty-four male Sprague-Dawley rats were divided into 3 groups equated for mean weight. Each group was then fed either standard pellets, restricted pellets, or a high-fat, high-carbohydrate diet until mean group weights diverged by 20%. The groups were designated "normal", "lean" and "fat", respectively. Animals from each group were then housed in boxes with running wheels and maintained on a LD 12:12 cycle (lights on 0500). After 8 days of ad lib feeding, food access was restricted to 2 hours per day (1100-1300) for 15 days. Each group was maintained on its pre-restriction diet. Restricted feeding was followed by 2 days of food deprivation. The experiment was conducted in two replicate runs.

Animals in the fat group developed AA on day 8 of RF, 4 days later than normal rats and 6 days later than lean rats. During the last 8 days of RF, the average number of wheel revolutions in the five hours immediately preceding food access was 49 for fat rats, 340 for normal rats, and 372.5 for lean rats. The level of AA in fat rats was significantly lower than in the other groups ($p < .001$, Mann-Whitney U test). The reduction in AA among fat rats cannot be attributed to lower overall activity, since nocturnal activity levels during the same segment of the experiment were not significantly different. During food deprivation, total activity increased within the normal and lean groups, but not within the fat group.

These results indicate that excess body weight induced by a high-fat, high-carbohydrate diet has a selective effect on the rate of development and the level of AA to restricted food access, and on the level of overall activity in food deprivation. Experiments in progress seek to determine whether diet or fat reserves are responsible for these effects.

61 Free-access to a running wheel alters both free-running period and brain monoamine rhythm in blinded rats.

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Our previous studies have shown that free-access to a running wheel shortened the free-running period in blinded rats. This fact together with those in other studies suggested that the free-running period of circadian system may be a subject to subtle enviromental influence. The aim of the present study was to confirm our previous results using young rats in large numbers as well as to determine brain monoamine rhythm to investigate the underlying mechanism. The free-running rhythm of male rats optically enucleated on the day of birth was measured for two weeks between 6 and 8 postnatal weeks with either an Automex (n=40) or a running wheel (n=61). Rats were sacrificed immediately after the termination the rhythm determination. Twenty-four hour patterns of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) contents in the following discrete regions in the brain were measured: frontal cortex (FC), striatum (ST), hypothalamus (HY), hippocampus (HC) and suprachiasmatic nucleus (SCN). Almost all (39/40) of rats subjected to the determination of locomotor rhythm with an Automex had a free-running period longer than 24 hr, while only 54.1% (31/61) of rats had a period longer than 24 hr. There was statistically significant difference in the mean of the free-running period between two rat groups ($p<0.001$). The running wheel group had significantly negative correlation between the free-running period and the revolutions per day ($R=-0.71$, $p<0.005$). NE rhythms in the FC, DA rhythms in the ST, HY and SCN and 5-HT rhythms in the FC and SCN showed signigicant difference between two groups (two way ANOVA). These results confirmed our previous findings and suggested two possible factors responsible for the change in the free-running period, i.e. activity of animale and monoamine metabolism.

62 LOCOMOTOR ACTIVITY AND LIGHT PULSES AS COMPETING ZEITGEBER STIMULI IN THE SCORPION CIRCADIAN SYSTEM.

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Circadian rhythms of the scorpion *Androctonus australis* were measured in long term experiments by means of simultaneous recordings of ERG amplitude and locomotor activity. In order to establish a PRC 4 hrs pulses of monochromatic light (LED, 555nm, 2000 lux at the lense of the eye) were applied to the median eyes.

The light pulses produce immediate phase shifts up to 5 hrs. Amount and direction of the phase shift, however, depend on the state of locomotor activity during the light pulse. The data pooled by this criterion - whether the Zeitgeber light causes increased activity or not - reveal two clearly distinct PRCs of almost the same shape and amplitude but shifted along the circadian time scale by about 9 hrs: The PRC from the normally active scorpion data looks very similar to every light-evoked PRC, whereas from the hyperactive scorpion data a PRC emerges which resembles the "non-photic-PRC" e.g. derived from induced activity pulses in hamsters (1).

So far, in scorpions induced activity alone (following 4 hrs lasting air puffs without additional light) causes a similar "non-photic-PRC" but with maximum phase shifts of about 1 hr.

These findings may help to discuss central information processing of light versus other Zeitgeber inputs. They as well may help to understand oscillatory processes in circadian rhythm research at least partly as results of feed-back loops: e.g. under constant conditions spontaneous changes of tau and phase occur when high activity coincides with peak times of the "non-photic-PRC".

(1) Mrosowsky, N. et al. (1989) Experientia 45: 696 - 702.

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HOW DOES DAYTIME LIGHT PREVENT DAMPING OF THE MELATONIN RHYTHM IN CULTURED CHICK PINEAL CELLS: REBOUND OR ENTRAINMENT?

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White light (L) has two effects on the circadian rhythm of melatonin output displayed by chick pineal cells in static culture: an acute suppressive effect on melatonin production, which can be blocked by the action of pertussis toxin (PT), and an entraining effect (indicated by its ability to cause phase shifts in subsequent cycles), which is not blocked by PT. Norepinephrine (NE) mimics the acute effect of L (and is blocked by PT), but not the entraining effect.

The rhythm of melatonin output damps more rapidly in constant red light (RR) than in a cycle of 12h white light and 12h red light (LR). This can be seen in the first cycle following a switch from LR to RR. Melatonin output is lower during the day in L than it is in R, but higher that night (in R) after daytime L than after daytime R. This effect might be due entirely to the entraining effect of L; the period of the rhythm is shorter in RR than in LR, bringing more of the output into the "day" and less into the "night". The entraining effect of L would also reduce the spread and lowering of "nighttime" melatonin output caused by divergence of phase in the population of oscillators present in each well. Alternatively, the higher nocturnal output after daytime L could be related to the acute suppression caused by L; it might be a "rebound" phenomenon. These alternative hypotheses differ in their predictions for the effects of NE and PT. If L works entirely by entrainment, then NE should not mimic and PT should not block this same-cycle effect of daytime L on nocturnal melatonin output. However, NE did mimic and PT did block this effect, suggesting that the ability of L to prevent damping is, at least in part, mediated by a same-cycle "rebound" effect following L's acute inhibition of melatonin production. Furthermore, NE enhanced the "rebound" effect of daytime L, suggesting a role for NE *in vivo* in regulating and maintaining the amplitude of the melatonin rhythm.

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PATTERNED LIGHT AND ENTRAINMENT OF LOCOMOTOR ACTIVITY IN MICE.

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Neurones in primary visual cortex show orientation-specific responses to patterns presented in the visual field and preliminary data recorded from suprachiasmatic (SCN) neurones in conscious rats indicates that SCN cells might respond in a similar way (Summerlee, unpublished observations). This raises the intriguing possibility that patterned-light stimulation may be important for entrainment to photoperiod. An experiment was devised to test this hypothesis. Mice of a CBA-Ca strain were maintained in total darkness and the free-running rhythm of locomotor activity determined. A pulse of polychromatic light was given for either 5, 20 or 40 min, 4 h after the onset of locomotor activity on day 8 of the free-running period and the phase shift determined. Response to the light pulse was compared in two groups of animals: group 1 were housed in cages with plain walls and white running wheels, group 2 were kept in cages with patterned walls and black running wheels. The 5 min pulse caused a non-significant ($P>0.05$) shift in the locomotor activity of the mice in the patterned cages only. Significant ($P<0.01$) shifts in the locomotor rhythm of both groups of mice were seen with 20 and 40 min pulses but with the 20 min pulse the shift was significantly ($P<0.05$) greater for the mice in the patterned cages (112 $\pm$ 13 min) compared with mice in the plain cages (60 $\pm$ 7). There was no significant ($P>0.05$) difference between the effects of the 40 min pulse for the two groups. The results suggest that patterned light may be important in the perception of light cues for entrainment of locomotor activity.

65 EFFECT OF MONOCHROMATIC LIGHT ON THE CHARACTERISTICS OF THE CIRCADIAN RHYTHM DURING THE ONTOGENY OF THE CRAYFISH. Fanjul-Moles, M.L°, Miranda-Anaya, M.* and Fuentes-Pardo, B.* . °Depto. de Biología, Fac. de Ciencias; *Depto. de Fisiología, Fac. de Medicina, U.N.A.M., 04510, México, D.F.

In the crayfish eye, spectral sensitivity changes during development (1). The aim of this work was to dilucidate whether these changes in spectral sensitivity influence the circadian rhythm development pattern of the electroretinographic (ERG) rhythm previously established with white light. Young crayfish aged between 1 day and 16 weeks after eclosion, and adult ones were used. ERG recordings were performed individually under darkness and constant temperature conditions for at least 9 consecutive days. During the first three days of experiments, the animals were submitted to test flashes of white light and from then on blue or red monochromatic light of the same intensity (200×10^{-4} Wm^{-2}) was used. Animals were their own witnesses, i.e., period, relative amplitude, and α/ρ ratio were measured for each period and the values obtained with white and with monochromatic light were compared. Very young animals (1-3 weeks) showed similar response patterns to white and blue light (high frecuency cycles prevailed); from the 4th to 8th weeks a circadian rhythm pattern appeared with maximal activity at night, wich is similar to that observed in the adult animal, although relative amplitude and α/ρ ratio were relatively high. No response to red light could be elicited in the animals younger than 6-8 weeks. However, after the 8th week the crayfish showed a subjective night similar to that shown by the adult animal with a circadian rhythm of 22.5 hs. and an α/ρ ratio of around 4. These results suggest an asymetric ontogenic development of two groups of photoreceptors with different spectral sensitivity, i.e., an early scotopic group and later photopic one. This asymetric development confers the ERG circadian rhythm different characteristics along the different stages of development.

(1). Fanjul-Moles M.L. and Fuentes-Pardo,B. (1988). Comp. Biochem. Physiol. 91A: 61-66.

66 ENTRAINMENT OF BEHAVIORAL CIRCADIAN RHYTHMS BY INFUSION OF PHYSIOLOGICAL LEVELS OF MELATONIN. C.C. Chabot and M. Menaker. Dept. of Biology, Gilmer Hall, Univ. of Virginia, Charlottesville, VA.

Melatonin has long been hypothesized to play an important role in avian circadian systems but direct evidence is lacking. In pigeons, recent work by Ebihara, Foa and their colleagues has provided limited support for this hypothesis. Here we report direct evidence from replacement studies. Blood was taken via an intravascular cannula (or by wing venipuncture) from both intact and pinealectomized (P-X) pigeons in constant darkness (DD) and their feeding and locomotor activity was monitored continuously. Blood levels of the pineal/retinal hormone melatonin were determined by radioimmunoassay. A syringe pump controlled by a timer was used to deliver 10 hour melatonin pulses, calculated to produce physiological levels in the blood, each day into P-X pigeons in DD via subcutaneous catheters. The phase of the circadian rhythms of feeding and locomotion of pigeons treated in this way was determined by the phase of the exogenous melatonin pulse. Furthermore, the pigeons' rhythms could be entrained by melatonin infusion cycles with periods either longer or shorter than 24 hours (23-25 hours). The blood melatonin levels of the infused birds were determined experimentally and were found to be within the physiological range of intact pigeons. These results suggest strongly that in the pigeon, and perhaps in other avian species, melatonin is an endogenous entraining agent for behavioral rhythmicity.

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AMBIENT TEMPERATURE INFLUENCES ENTRAINMENT OF SIBERIAN HAMSTER ACTIVITY RHYTHMS. Elena M. Thomas, Department of Psychology, University of California, Berkeley, CA 94720.

Twenty-four adult, gonadectomized male hamsters were housed in an LD 16:8 photoperiod in cages equipped with running wheels at an ambient temperature (Ta) of 23°C. After 5 weeks, Ta was lowered to 13°C for 5 weeks and finally increased to 23°C for a further 5 weeks. During the 3rd to 5th week of exposure to Ta=13°C, 21 out of 24 hamsters showed advances in the onset of wheel running activity; mean change in phase angle difference (PAD) was an advance of 43±11 minutes (n=24). During the 3rd to 5th week of re-exposure to Ta=23°C, 17 out of 24 animals showed delays in activity onset; mean change in PAD was a delay of 5±11 minutes (n=24). These significant changes in PAD did not appear until 1 to 3 weeks after the change in Ta. In contrast, activity levels increased on the first night after a decrease in Ta and decreased with similar latency after an increase in Ta. Body temperature (Tb) was monitored throughout the experiment via intraperitoneal transmitters (Minimitter Inc.); the change in PAD was not significantly correlated with changes in mean Tb.

The data indicate that changes in ambient temperature influence the timing of activity onset, presumably by affecting the period of an underlying circadian oscillator. These results document the temperature-dependence of mammalian pacemakers.

68 **THE ENTRAINMENT OF CIRCADIAN ACTIVITY RHYTHMS TO FEEDING SCHEDULES.** H. Abe* and S. Ebihara, Dept. Aerospace Psychol., Res. Inst. Env. Med., Nagoya Univ., and Dept. Animal Physiol., Nagoya Univ., Nagoya 464-01, Japan. *Present address: Dept. Psychol., Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

CS mice are an inbred strain which was established at Nagoya University. We have found that these mice show a daytime peak in activity at around the time of food replenishing. This finding raises the possibility that the circadian pacemaker in CS mice would be affected by restricted feeding schedules (RF). In Syrian hamsters, Rusak et al. reported that a periodic hoarding opportunity could entrain circadian activity rhythms. This also raises the possibility that their circadian pacemaker is entrainable to RF. We report here the results of studies investigating the effects of RF on circadian activity rhythms in CS mice and hamsters. In the CS study, the food cup was put into each cage for 2 hr. at a fixed time of day (RF). During RF, stable phase relationships between the activity rhythms and RF were established. After the completion of the RF, the rhythms freeran from phase expressed during RF. The effects of RF were also studied in suprachiasmatic nucleus (SCN) lesioned animals. While the activity was arhythmic during *ad lib* feeding after SCN lesions, food anticipatory activity was observed during RF. In the hamsters, a modified method for RF was used since they cannot survive under temporally restricted feeding schedules. We gave each animal food pellets in the amount of 70% of the hamster's mean daily food intake at a fixed time of day (70% RF). During 70% RF, stable phase relationships between rhythms and the phase of food presentation were established in some animals. The rhythms freeran after RF from the phase expressed during RF. These results suggest that in CS mice and hamsters, the circadian pacemaker in SCN may be entrained to feeding schedules, and that there may be a tight coupling relationship between the SCN and the food entrainable oscillator (FEO) .

69 THE ROLE OF THE LATERAL HYPOTHALAMIC AREA (LHA) IN THE REGULATION OF HAMSTER LOCOMOTOR RHYTHMS. N.L. GOODLESS-SANCHEZ, R.Y. MOORE and L.P. MORIN. Depts. of Psychiatry and Neurology, SUNY Stony Brook, Stony Brook, NY 11794. The retinohypothalamic tract projects to the lateral hypothalamic area (LHA). The functional significance of this path is not known, but it does suggest that the LHA may play a role in rhythm regulation. Previous research has reported both an increase in the level of activity and duration of the active phase of wheel running behavior after lesions of the primary optic tracts. Review of this literature does not clearly reveal whether or not the lesions included or damaged the LHA. The present lesion study was designed to specifically examine the role of the LHA in the regulation of hamster locomotor rhythms.

Male golden hamsters received bilateral electrolytic lesions directed at the LHA. Animals were subjected to different light cycles for approximately 5 months. Lesions produced immediate and sustained advances of the onset of running activity ($t=5.07$, $p<.001$). This new advanced phase angle of entrainment was maintained during all LD cycles. Alpha was also increased in experimental animals compared to controls ($\bar{X}=8.33$ hrs $\pm$ 1.73, $p<.001$). However, the activity level, measured in revolutions/day remained constant before and after surgery. Phase shifting rates in response to a 6 hr phase advance or phase delay were similar for the two groups. Finally, the circadian period measured in constant dim illumination for the two groups remained similar throughout the study.

These changes in activity onset and offset and duration of wheel running behavior suggest a role for the LHA in circadian rhythm function. The data are consistent with work previously done on our laboratory. The distribution of nocturnal activity by LHA lesioned, 5,7-DHT treated or parasagittal knife cut (near the SCN) animals is relatively similar. These manipulations advance the onset of wheel running activity and delay the offset. In each case the observed phase and duration changes may have resulted from damage sustained by 5-HT fibers ascending to the SCN within the medial fiber bundle.

70 THE ROLE OF EFFERENT PATHWAYS IN PHASE-SHIFTING CIRCADIAN RHYTHMS OF WHEEL-RUNNING IN THE GOLDEN HAMSTER. M.E. Harrington and T. Rahmani, Department of Psychology, Smith College, Northampton, MA 01063.

When phase-shifted by light, behavioral rhythms require several cycles before a steady-state phase shift is accomplished. In a brain slice preparation of the suprachiasmatic nuclei (SCN), the phase shift caused by a light pulse appears to be fully accomplished in the first cycle (Gillette, Brain Res., 379 (1986) 176-181). This disparity between behavioral and electrophysiological measures might be due to some aspect of the damage inflicted by preparation of the brain slice. This experiment was designed to determine if the rate of entrainment to a light:dark (LD) schedule could be increased by lesions of specific output pathways of the SCN.

Twenty-four male golden hamsters were housed in a colony room (LD 12:12; lights on 0700-1900). Hamsters received either lesions (n=16), sham surgery (n=4) or no surgery (n=4) and were placed into individual running-wheel cages under LD 14:10 (lights on 0600-2000 h). The LD schedule was phase advanced by 6 h, phase delayed by 6 h, and the animals were exposed to constant dim light (4 weeks per condition). At the end of the experiment, coronal brain sections were processed for peptide histidine isoleucine (PHI) and bombesin immunocytochemistry. Every third section was stained with the Kluver-Barrera method for cells and fibers.

One hamster showed a faster rate of re-entrainment than control animals after a phase advance of the LD cycle. This hamster had a lesion of the dorsal half of the SCN and the periventricular area dorsal to the SCN. Four hamsters showed selective damage to the rostral efferent pathway of the SCN. These animals took approximately twice as long as controls to entrain to the first LD schedule. In one case rhythmicity was severely disrupted for 4 weeks. Two hamsters with extensive SCN damage showed arrythmicity, followed by recovery of rhythmicity after several months. These hamsters had only two or three PHI-immunoreactive fibers emerging from the lesion site.

These results indicate that: (1) neural circuits disrupted by damage to the dorsal SCN area may play a role in slowing phase shifts of behavioral rhythms, (2) damage to the rostral path of SCN efferents can disrupt entrainment and rhythmicity, and (3) very few SCN efferent fibers may be necessary for the expression of circadian rhythmicity.

71 IMITATIONS OF THE CIRCADIAN CHANGES IN RABBIT PHOTIC RESPONSES, ELICITED BY STIMULATION OF THE CERVICAL SYMPATHETIC NERVES OR SUPRACHIASMATIC NUCLEI. A.C. Bobbert, F. Eggelmeijer, and J.J. Riethoven. Department of Physiology and Physiological Physics, Leiden University, Leiden, The Netherlands.

Rabbits which have been exposed for several weeks to the natural sequence of daylight and nocturnal darkness, or to fixed 24-h L:D alternations, exhibit afterwards a "programmed" rhythm in retinal sensitivity to flashes and steady illumination. This rhythm consists of a phase in which the animals respond to flashes with "Day Time Potentials" (D.T.P.'s) for the ERG's and cortical visually Evoked Potentials (V.E.P.'S), and a phase with "Night Time Potentials" (N.T.P.'s) reflecting a raised photic sensitivity of the retina.

It now appears, from a comparison between the circadian fluctuation in the ERG's and V.E.P.'s and their changes induced by low-frequency stimulation of the cervical sympathetic nerves, that the latter contain fibres which discharge rarely, if at all, in the D.T.P.-phase and fire at the low rates of 2 cps or less during the N.T.P.-phase -- and evoke in this way the rhythm in retinal sensitivity.

It further appears that identical conversions of the ERG from D.T.P.'s into N.T.P.'s can be induced by focal stimulation in the close vicinity of, or inside the suprachiasmatic nuclei.

These results give further evidence for the presence in rabbits of a retino-hypothalamo-retinal loop with a 24 hours-delayed feedback.

72 SHORT TAU(DD) IN ALBINO MUTANT MICE: ABSENCE OF A NORMAL AFTER-EFFECT? Bernard Possidente, Carol Lyons, and Elizabeth Carlson. Biology Department, Skidmore College, Saratoga Springs, NY 12866

There are scattered reports of an apparent shortening of tau(DD) in albino mice of various genetic backgrounds. Here we show that an albino mutation, isolated against an isogenic inbred C57BL/6J strain background, is associated with an approximately 30 minute shortening of tau(DD) in mice that were tested at age six months. The effect persisted for approximately 100 days of testing. A second experiment, using mice of the same genotypes but beginning at age two months resulted in a similar difference in tau(DD) that disappeared after about four weeks as the pigmented mice gradually shortened their period down to the albino value. These results suggest that the albino effect on tau(DD) may be mediated through the absence of a normal after-effect with an age dependent decay rate. All mice were bred, raised and maintained on a 16:8 LD cycle before testing. A second pigment mutation, pinkeye-dilute, which results in an albino eye but dilute pigmentation in the rest of the body, had no effect on tau(DD) in isolation against an isogenic inbred C3H/HeJ background. The interpretation of this result is complicated by the fact that this inbred strain is fixed for a recessive mutation causing retinal degeneration.

73 EFFECTS OF THYROIDECTOMY AND ORCHIDECTOMY ON CIRCADIAN RHYTHMS OF WHEEL RUNNING ACTIVITY IN RATS ON A LIGHT-DARK CYCLE. J.E. Ottenweller, W.N. Tapp, and B.H. Natelson. Neurobehavioral Unit (127A), VA Medical Center, East Orange, NJ 07019 and Department of Neurosciences, New Jersey Medical School, Newark, NJ

Male rats were randomly assigned to 4 groups: 1) sham-thyroidectomized (thyrex) and sham-orchidectomized (orchidex), 2) thyrex and sham-orchidex, 3) orchidex and sham-thyrex, and 4) thyrex and orchidex. After surgery, rats were placed on 1% calcium chloride drinking water to counteract parathyroid removal in thyrex rats. Ten weeks after surgery, 6 rats in each group were placed in Wahmann running wheels for 3 weeks under a 12:12hr light-dark cycle (onset of light: 0600h). Wheel turns were collected in 10 min bins by a computer and then collapsed into 3 hr bins for analysis. Thyrex produced an overall increase in running ($P < 0.05$), whereas orchidex did not. Periodic regression was used to estimate the amplitudes and phases of the activity rhythms. Thyrex increased the amplitude of activity rhythms ($P < 0.05$), but it is possible this was due to the overall increase in activity seen in thyrex rats. If activity was expressed as a percent of total daily activity in each 3 hr bin, the amplitude of activity rhythms in thyrex rats was actually lower than in sham-thyrex rats ($P < 0.05$). Thus it remains unclear how thyrex affected the amplitude of activity rhythms in this study. Orchidex did not affect the amplitudes of activity rhythms. There were no effects of either surgery on the phases of the sine curves fitted to the activity rhythms. Thyrex of sham-orchidex rats decreased the variability in amplitude and phase estimates which came from day-to-day variability in these parameters within each animal. However, orchidex seemed to block this decrease in day-to-day variability caused by thyrex. In summary, thyrex affected the amplitude of activity rhythms and their day-to-day variability, but orchidex appeared to have no prominent effects in rats maintained under light-dark cycles. Supported by VA Medical Research Funds.

74 Li Jing Cai, Wang Ai Min and Li Cheng Ji
Department of Physiology, Shenyang College of Pharmacy, Liaoning, China
CIRCADIAN RHYTHMICITY OF SERUM CORTICOSTERONE OF INTACT AND PINEALECTOMIZED MICE*

The circadian levels of serum corticosterone were determined in intact and pinealectomized male mice which have been acclimatized in a 12:12 h light:dark cycle for two weeks. Results of this experiment show that serum corticosterone of pinealectomized mice exhibited in a similar circadian pattern to that of normal mice. However, the corticosterone concentrations of the former increased approximately by three times as compared with that of the later at each timepoint tested.

Administering exogenous melatonin to mice resulted in decrease of serum corticosterone in pinealectomized but not in intact. Iv morphine hydrochloride manifested an increase of melatonin release from the pineal and a decrease of serum corticosterone in the dark phase in normal mice.

The obtained data suggest that pineal gland is not evident action on the circadian rhythm of corticosteroid but it has an important role in the regulation of hypothulamus-pituitary-adrenocortical axis in the mice.

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EEG DELTA ACTIVITY IN SQUIRREL MONKEY SLEEP DEPENDS ON CIRCADIAN PHASE AND LENGTH OF TIME AWAKE

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The delta frequency component (0.5-2.0 Hz.) of the EEG during NREM sleep has been proposed as a physiological correlate of a process or substrate which accumulates during wake and decreases during NREM sleep. In these models, circadian factors play only an indirect role, primarily by influencing the timing of sleep. In most relevant studies, however, sleep was allowed only during the inactive phase of the circadian cycle or after wake durations of less than 24 hours, thereby confounding circadian phase and length of time awake.

We have examined EEG delta activity in squirrel monkeys (*Saimiri sciureus*) during undisturbed sleep in LD and LL, and following sleep deprivation (SD) in LL. Six different SD lengths were studied; all began at the time of predicted consolidated sleep (CS) onset and ended 0, 1/4, 1/2, 1, 1-1/4, or 1-1/2 circadian cycles later. Both Fourier Transform (yielding power density values) and zero-crossing (yielding wave incidence and wave amplitude values) analyses were performed on the EEG data to study delta activity.

In LD and LL, all delta activity measures exhibited circadian rhythms, with maximal values approximately 4 hours after CS onset. In agreement with previous reports of increased delta activity with increased time awake, the ratio of post SD to baseline values was larger ($p=0.05$) for SDs longer than one cycle (1, 1-1/4, 1-1/2 cycles) compared with those shorter than one cycle (0, 1/4, 1/2 cycles). However, delta activity was not a monotonically increasing function of wake duration . Delta activity ratios was higher after SDs ending mid-subjective night (1/4, 1-1/4 cycles) than after SDs ending at the beginning (0, 1 cycles) or end (1/2, 1-1/2 cycle) of subjective night ($p=0.05$). Furthermore, the largest post SD ratio was seen immediately after the 1-1/4 cycle SD, at approximately the same phase as the largest baseline values in undisturbed LD and LL conditions. Thus, both circadian phase and length of time awake affected the delta activity in the EEG.

76 CIRCADIAN RHYTHMICITY AND REPRODUCIBILITY IN NUCLEIC ACIDS SYNTHESES OF SEVERAL MURINE TISSUES.

Wang Ai Min, Li Jing Cai, Wang Yukun, Ma Kongchen, Wang Min and Ge Shu. Department of Physiology, Shenyang College of Pharmacy, Liaoning, China.

These studies were done with mice and rats that had been standardized to 12 h light alternating with 12 h darkness to determine the circadian rhythm in DNA and RNA syntheses of liver, spleen and kidney as well as to go further into thair affecting factors.

Circadian variation of nucleic acids: DNA and RNA were determined at six timepoints over a 24-h span in mice. A marked circadian rhythm was noted, the peak value of DNA and RNA occured at 02:00 h and valley occured from 10:00 to 14:00 h in kidney. The phase of nucleic acids in spleen was slightly late than that of kideny, peak at 06:00 h, valley at 14:00 h. The difference between peak and vally are statistically significant, $P<0.05$ in each case.

Reproducibility of the phasing of the rhythm: The levels of DNA and RNA in rat liver were determined in April for three years running with same techniques. Results of this experiment show that both DNA and RNA are biphasic rhythm. DNA first peak occured at 08:00 h (the beginning of light), second peak occured at 24:00 h (middle dark phase). The two peak in liver RNA was 4 h later than DNA. The remarkable reproducibility of nucleic acids circadian rhythm were found from 1984 to 1986, it is evident that the phasing of these rhythm is steady.

Influence of epinephrectomy on DNA and RNA: It has been found that the circadian levels of DNA and RNA decreased in epinephrectomized adult mice and the circadian pattern of those were changed. The level of nucleic acids were restored after hydrocrotisone administration.

77 RESEARCH ON RHYTHMS IN RABBITS

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There appears to be some confusion concerning the question, whether the laboratory rabbit is a diurnal, a crepuscularly active or a nocturnal animal. We like to present some typical chronobiological characteristics of rabbits, kept in an isolation unit during an LD 12:12 and in the absence of any zeitgeber.

In addition, we present some simple explanations for equivocal results, reported in the literature as mentioned above.

Details on following items will be given:

1. circadian rhythms of locomotor activity, food intake, water intake, hard faeces excretion and of urine excretion during entrainment with LD 12:12 and in the absence of a zeitgeber;
2. unimodality and bimodality of circadian rhythms;
3. effect of animal house activities on circadian rhythms;
4. effect of scheduled feeding on circadian rhythms.

Conclusion: kept in an isolation unit, the rabbit exhibits clear circadian rhythms. So far, different functions of an individual did not desynchronize internally. While there is some activity during the light time, the rabbit is a predominantly nocturnal animal.

However: exposed to animal house activities or in consequence of feeding the animal exclusively during the light hours the rabbit can be turned to a predominantly diurnal animal.

78 TIMING OF BIRTH IN HAMSTERS. N. Viswanathan and Fred C Davis, Department of Biology, Northeastern University, Boston, MA 02115.

A fetal circadian clock begins to oscillate prenatally and is entrained by maternal rhythms. The functional significance of this early development and entrainment is not understood. We report here, the results of preliminary experiments examining one possible function, the fetal timing of birth. Hamsters maintained in LD 14:10 h were mated at two different times on the day of ovulation. On day 14 of gestation they were placed in dim LL and observed every hour until birth. The majority of births occurred during the subjective day on day 16 but at two different times correlated with the different times of mating. In addition, the larger litters were born earlier than the smaller litters. Interestingly no births occurred during the early subjective night suggesting the involvement of circadian control in the timing of birth. To examine this possibility, two groups of hamsters were mated at one time of day but the light/dark cycle was either advanced or delayed for each group by 6 hours between days 5 and 14 of gestation followed by dim LL. The time of birth was significantly different between the two groups, demonstrating a role for circadian timing. The delayed group gave birth an average of about 8 hours after the advanced group. From these results we suggest that both developmental and circadian timing influence the time of birth. If a fetal clock is involved in the circadian timing, it is possible that a mutation of the fetal clock will affect the time of birth. We examined this question using a wildtype mother carrying fetuses heterozygous for the τ_s mutation. Although the time of birth was the same for these fetuses as for wild type, this does not exclude a role for the fetal clock in the timing of birth. Supported by NIH grant HD 18686 to FCD.

79 TIMING OF DEVELOPMENT OF THE CIRCADIAN CLOCK CONTROLLING THE RELEASE OF SPERM FROM THE TESTIS OF A MOTH.

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Release of mature sperm from the testis into the seminal ducts of the gypsy moth occurs in a daily rhythmic pattern. The circadian system which controls this rhythm and the photoreceptor which determines its phase are both located within the testis - seminal duct complex. Rhythmic release of sperm is initiated between 8 and 9 days after pupation. We attempted to determine when the clock underlying this rhythm develops. Insects that were reared since pupation in constant darkness (DD) subsequently released sperm in a non-rhythmic fashion. Release of sperm could be made rhythmic by exposing DD pupae to a single 8h pulse of light or a high temperature 6 or 7 days after pupation but not 4 or 5 days after pupation. Thus, the circadian mechanism controlling the rhythm of sperm release begins to function approximately 2 days before the release is initiated. At that time a significant decline occurs in the blood titer of the hormone, 20-hydroxyecdysone. Injection of this hormone into pupae caused inhibition of sperm release but did not seem to affect the circadian mechanism which controls the timing of the release. 20-hydroxyecdysone could prove useful in biochemical study of this system for temporal separation of the clock and its output.

80 CIRCADIAN RHYTHM OF CHEMOTAXIS IN CHLAMYDOMONAS. Edward Byrne and Carl Hirschie Johnson*. Dept. of Biology, Middle Tennessee State University, Murfreesboro, TN, 37132, and *Dept. of Biology, Vanderbilt University, Nashville, TN, 37235.

Previous work by Bruce has shown that cells of the alga Chlamydomonas reinhardtii express circadian rhythms of phototaxis and "stickiness" to glass. In the search for other rhythms in Chlamydomonas which are controlled by the circadian pacemaker, we have found that chemotaxis towards ammonium is rhythmic. Chemotaxis was assayed in darkness by counting cells which swim into capillaries filled with medium containing ammonium as compared with into control capillaries filled with ammonium-deficient medium. Chemotactic responsiveness is rhythmic when assayed at various phases during light/dark cycles or in continuous conditions (e.g., DD). The rhythm persists for at least 3 cycles in constant conditions, although its amplitude damps significantly. Chemotactic responses peak at mid- to late-subjective night, whereas the phototaxis rhythm peaks in the early subjective day. Other studies have shown that the rhythm of phototaxis cannot be due merely to daily differences in generalized motility (Pfau et al, Arch. Microbiol. 135: 259-264; Kondo et al, Protoplasma [Suppl. 1]: 185-192). Likewise, microscopic assessment of cell motility demonstrates no major differences in motility between cultures which do express large differences in chemotaxis. Therefore, chemotactic efficacy seems to be specifically modulated by the circadian pacemaker. To our knowledge, this is the first report of circadian chemotaxis in any organism.

81 STATISTICAL ANALYSIS OF ULTRADIAN RHYTHMS: A COMPARISON OF DIFFERENT METHODS OF TIME SERIES ANALYSIS. U. Siebert and F. Wollnik, Department of Biology, University of Konstanz, D-7750 Konstanz, F.R.G.

The statistical analysis of biological time series can provide useful and necessary quantitative descriptions of biological rhythms. However, since most methods of period analysis were originally described for the detection of circadian rhythms, their applicability to ultradian rhythms (shorter than 24 h) is still questioned, especially if the ultradian rhythms are superimposed on circadian rhythms.

We have tested the usefulness of 4 methods for detecting ultradian rhythms in biological time series: autocorrelogram, power spectrum analysis, periodogram analysis and maximum entropy spectral analysis (MESA). To test their applicability and their results the following types of data have been used: (1) artificial data with ultradian and circadian rhythms of known wave form, period, and amplitude, (2) biological data from activity recordings of laboratory rats, cotton rats, common voles, and flies.

The results show that power spectrum, periodogram and MESA are equally useful methods for detecting the presence of ultradian rhythms. In the power spectrum, the amplitudes of spectral estimates are correlated with the strength of a given rhythm; differences in the amplitude can be confirmed with further statistical tests. However, this method can detect only certain periods due to its limitation to integer frequencies. Periodogram and MESA, on the other hand, detect the entire range of periods given by the length and resolution of the data sample. Furthermore, the periodogram allows to assess the statistical significance of rhythmic components. We recommend that an analysis of biological time series be based on at least two of the above methods in order to compensate for limitations of each individual method.

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82 MASCULINIZATION OF FEMALE SLEEP CYCLE RHYTHMICITY BY PERINATAL INJECTION OF TESTOSTERONE. W. Fishbein, J. Fang, S.W. Yang and S.J. Tien. Neurocognition Program, CUNY, City College & Graduate School, NY 10031.

In 1987 we reported for the first time, in the mouse, that the ultradian cyclicity of sleep is sexually dimorphic. At the same time we also reported that prenatal stress completely sex-reversed the sleep of males. Since prenatal stress produces a reduction in fetal testicular enzyme activity with an accompanying reduction in plasma levels of testosterone, we concluded that sleep-cycle rhythmicity follows the same general rule as the genital system: the basic plan is female.

We subsequently extended our observation of sexual dimorphism to the Sprague-Dawley rat. A key finding, in both species, is that the sexual dimorphism of sleep is solely accounted for by a sex-linked difference in the frequency of paradoxical sleep (PS) bouts. It appears that the normal biological clock timing mechanism(s) that controls the interval between PS episodes runs at a different speed in males than in females. As a result the total amount of PS is substantially different between the sexes.

Since testosterone might be the catalyst of this sex linked difference, we undertook the present experiment to determine whether female sleep cyclicity is subject to masculinization by perinatal injection of testosterone (1,000ug/50ul/animal injected < 24 hours after birth).

The baseline data replicates our previous observation of sex differences; namely, control (safflower oil, 50ul) injected male rats exhibit more PS bouts than control females. Whereas perinatal testosterone loading in males had no effect, perinatal testosterone injected females display significantly more PS bouts than control females. The sleep cyclicity of testosterone injected females was indistinguishable from the vehicle and testosterone loaded males.

The results provide strong evidence that the sleep (PS-Slow Wave Sleep) cycle ultradian clock is genetically organized, but remains susceptible to modification by male gonadal steriods up to the time of birth.

82A

Nikola S. Kovacevic and Ivan M. Milosevic

DIURNAL RHYTHM INFLUENCING LEARNING IN THE MARINE FISH *Serranus scriba* CUV.

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Ecological features of *Serranus scriba*'s biotop do deeply influence both its ethology in its natural setting and its behavior in the laboratory. We have found that gross motor activity remains maximal during the evening hours and decreases during the day also under laboratory conditions.

We did analyze systematically, the circadian dependence of different types of learning phenomena which might be relevant from the ecological and ethological points of view.

Avoidance conditioning was performed using two types of shuttleboxes (with electric shock and tactile stimulation as unconditioned stimuli and light as conditioned stimulus). Experimental sessions took place at about 8 a.m. - morning, 12- noon and 16p.m.- evening, for 21 days either successively in the same fish or the fish were trained separately only in the morning, noon or evening. It was a general finding that the above 90% criterion of correct responses is reached earlier and their incidence is higher under evening conditions and in the evening group in comparison with the remaining ones. In case the fish is for 7 days and trained further under constant light or dark condition circadian differences in learning disappear. That happens, however, at the level of their best performance.

In other experiments alimentary conditioning was performed in 3 separate groups of fish in the morning, noon, evening, the conditioned stimulus being a triangular pattern lightened by a lamp. This type of learning required about twice as much trials in comparison with avoidance. The criterion of above 90% of correct responses, likewise, was reached earlier, and their incidence was higher, in the evening group again in comparison with the others. The evening group demonstrated also better performance in differentiation between two visual patterns, the triangle signaling food and a circle signaling electric shock. Both the defense conditioned reflex developing first, and the reappearance of the alimentary reaction following after, were facilitated in the evening group.

The above results are probably related to increased activity, including feeding, in the evening under natural conditions.

83 ALTERATIONS OF ACTIVITY RHYTHMS OF RATS RECEIVING TRANSFECTED CELL LINES. A Morris, B Tate-Ostroff, RE Majocha, and CA Marotta. Neurobiology Laboratory, Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Alzheimer's disease is characterized by disruption of circadian rhythms. Genetically engineered cell lines that overexpress the A4 region of the amyloid precursor protein (APP) provide a novel tool for the development of an animal model of amyloidosis of Alzheimer's disease and its effects on circadian rhythms. Transformed pheochromocytoma (PC12) cells were prepared using amyloid cDNA corresponding to the A4 to C-terminal region of the APP. Transfection was also performed with frame-shifted amyloid cDNA. Both cell types were stereotaxically implanted into the suprachiasmatic nuclei (SCN) of rat brains. After surgery, activity data were collected via a model VM-FH Minimitter transmitter implanted intraperitoneally. The strength of an animal's 24-hour rhythm was determined using power spectrum analysis for a period 6-8 weeks post-surgery. The mean strength for each surgical group was computed and compared by analysis of variance. Implants of both cell types significantly ($p<.001$) disrupted activity rhythms as compared to unoperated controls. Histological examination of the brains revealed that the nuclei retained their integrity. We conclude that transfected PC12 cells severely disturb activity rhythms when implanted in the SCN and that the causes of this disturbance merit further investigation. Supported by grants P01AG02126 and a Metropolitan Life Foundation Award (CAM); and a Milton Fund Award (REM).

84 FREE-RUNNING RHYTHMS, THE ENDOGENOUS COMPONENT OF TEMPERATURE AND THE EFFECT OF LIGHT INTENSITY ON CIRCADIAN RHYTHMS IN YOUNG AND OLD RATS

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Several studies have reported alterations in the period of free-running rhythms (FR) during aging. However, as the level of activity directly influences the FR period, and old rats are always less active than young ones, the reported findings need re-examination. By comparing the temperature, drinking and sleep/wake rhythms instead of wheel running in young (3-5 months) and old (30-32 months) Brown-Norway rats, we found no differences either in the total amount of active wakefulness, or in period of FR during aging. There was also no sign of internal desynchronization among the various rhythms.

A mathematical approximation method was used to estimate the endogenous component in the FR of temperature data (correcting for the amount of active wakefulness). The amplitude of the endogenous component did not differ between young and old rats. In contrast, the masking effect of activity on body temperature was significantly higher in young rats.

Under entrained conditions old rats have been reported to show a reduced amplitude of various circadian rhythms. As perception of light may directly influence the level of activity we have studied the effect of 5 different light intensities (ranging from 445 to 3.5 lx) during the light phase of the LD cycle in young and old rats. As predicted, reducing the light intensity had a significant negative effect on the amplitude of the sleep/wake rhythms in both age groups. Furthermore, a reduced amplitude in old as compared with young rats was seen at all light intensities. The amplitudes of old rats under the highest light intensity were comparable to those of young rats under the lowest light intensity. We conclude that amplitude reductions of circadian rhythms during aging can be compensated for by increasing the environmental light intensity.

85 STIMULATED ACTIVITY INDUCED BY TRIAZOLAM OR DARK PULSES DOES NOT PHASE SHIFT THE CIRCADIAN CLOCK OF OLD HAMSTERS.

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We have previously reported that the circadian clock of old hamsters remains sensitive to the phase advancing and phase delaying effects of light. Since little is known about the effects of aging on the response of circadian clocks to other phase shifting stimuli, we compared the ability to phase shift the circadian activity rhythm of young (2-5 months) and old hamsters (19-28 months) with 1) injections of the protein synthesis inhibitor, anisomycin, 2) injections of the short-acting benzodiazepine, triazolam, or 3) presentations of 6-h dark pulses. In young animals, these three stimuli generate a similar type of phase response curve (PRC) different from the PRC to light. Dark pulses and triazolam but not anisomycin are thought to induce phase shifts by inducing an acute change in the arousal state and/or an increase in locomotor activity.

Results of the experiment with anisomycin indicate that old animals were still responsive to the phase advancing and phase delaying effects of this protein synthesis inhibitor. On the contrary, while dark pulses or triazolam induced similar increases in locomotor activity in young and old hamsters, no phase advances or delays were observed in the activity rhythm of old hamsters in response to these stimuli. Our findings indicate that while the circadian system of old animals remains responsive to the phase shifting effects of some stimuli (e.g. light, protein synthesis inhibitors) with advancing age, there is a total loss of responsiveness to the phase shifting effect of activity inducing agents. This may prove to be an interesting model to stuoy the effects of age on circadian clocks. Contrat "Sciences de la vie" B10/04 du Ministère de la Politique Scientifique.

* Senior Research Assistant of the FNRS (1989-1991).

86 CIRCADIAN PROFILES OF CORTISOL AND TSH DURING SLEEP DEPRIVATION IN ELDERLY MEN.

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We have previously shown that aging is associated with decreased amplitude and advanced timing of the diurnal variations of cortisol and TSH. However, the secretion of of both these hormones is influenced by sleep. Since, in the elderly, sleep duration and quality are decreased, the alterations in the diurnal profiles could at least partially reflect these sleep perturbations. Our aim was to delineate the relative roles of sleep and circadian rhythmicity in modulating cortisol and TSH secretion in the elderly. Eight normal young men, ages 22-27 yrs, and seven healthy older men, ages 59-72 yrs, were studied over a 57-h period including a baseline period with sleep during normal bedtime hours (23-07) and a complete 24-h cycle with total sleep deprivation. All studies were preceded by two nights of habituation. To avoid confounding effects of meals, caloric intake consisted of a constant glucose infusion at a rate of 5g/kg/24 hrs. Sleep was monitored. Blood sampling for cortisol and TSH was performed at 20-min intervals throughout the study. TSH assay is currently under progress. Diurnal variations of cortisol levels were quantified using an algorithm calculating a polynomial regression line which was used to accurately estimate the amplitude of the rhythm and the timing of the nocturnal nadir. Mean ± SD of cortisol results are:

	baseline			sleep deprivation		
	young	old	p	young	old	p
amplitude (µg/dl)	5.8 ± 1.1	4.3 ± 1.0	<0.01	5.2 ± 1.2*	3.8 ± 1.6	<0.05
timing of nadir	23:55 ± 44m	22:26 ± 67m	<0.02	23:05 ± 68m	21:53 ± 67m	0.06

* $p<0.05$ as compared to baseline.

Thus, in the elderly, advanced timing and diminished amplitude of the circadian rhythmicity of cortisol secretion are independent of sleep. These data suggest that the circadian signal is both advanced and dampened in old age.

87 CIRCADIAN RHYTHM DISORDERS OF SLEEP-WAKING, BODY TEMPERATURE AND MELATONIN SECRETION IN ELDERLY-PATIENTS WITH DEMENTIA AND THEIR PHOTOTHERAPY

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Many elderly patients with dementia are known to have disorders of the sleep-wake rhythm and behavioral disorders including nocturnal delirium. We examined the circadian biological rhythms in such patients in order to find the aging effects on the biological rhythm and its relation to the behavioral disorders.

The subjects consisted of 16 patients with dementia (11 with multi-infarct dementia and 4 with senile dementia of Alzheimer's type), aged 56-89, and 7 patients without or with dementia of a slight degree, aged 65-81, as a control group.

The sleep-wake states of the patients were frequently observed by nurses for 1-4 months. The oral or rectal temperature was recorded for 3-7 consecutive days. Serum melatonin was assayed in 10 patients. Blood was sampled two times a day at 0000 and 1200 hours. For the treatment of sleep-wake rhythm and behavioral disorders, phototherapy with illumination of 2,500 lux was performed every morning for 2 hours during 1-3 months.

Results were as follows: All the 16 patients showed a sleep-waking rhythm disorder, 8 of them (50%) showed a body temperature rhythm disorder and 6 of the 10 patients (60.0%) showed a disordered melatonin-secretion rhythm (reduced amplitude). For the treatment of sleep-waking rhythm disorder, phototherapy was effective in 8 of the 16 patients (50.0%). However, disordered body-temperature rhythm improved in only 3 of the 16 patients, and disordered melatonin rhythm never improved during the phototherapy. Six of the 16 patients did not respond to any of the treatments.

88 SLEEP IN HEALTHY 80 YEAR OLDS FOLLOWING A 6-HOUR PHASE ADVANCE IN ROUTINE

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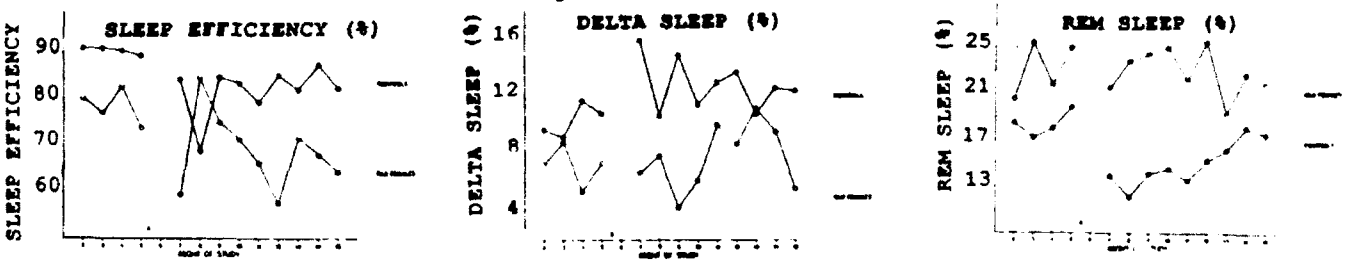
Aging is accompanied by changes in sleep continuity, delta sleep, and REM sleep timing in humans, which could be due to weakening of homeostatic or circadian sleep regulatory mechanisms. To address this issue, we examined EEG sleep variables in elderly subjects after an acute phase shift in routine.

Five healthy 80 year-old women completed a 15-day time isolation study, with continuous core body temperature measurement, mood and performance tests every 2 hours, and sleep EEG recordings each night. Subjects strictly followed their habitual 24-hour routine for 5 days, with complete darkness during the in-bed episode, <500 lux during wakefulness, strict meal times, and no napping. On night six there was an unheralded 6-hour phase advance of wake time, truncating the sleep episode. The routine continued at this earlier phase for the remainder of the study. Control subjects were 8 middle-aged men studied under identical conditions.

Compared to controls, elderly subjects had impaired sleep efficiency and delta sleep after the phase shift, but REM sleep, like rectal temperature, appeared to entrain more rapidly. These results suggest that age-related sleep changes in humans are more likely due to impaired homeostatic regulation (reflected in sleep continuity and delta sleep) than to impaired circadian rhythm generation or entrainment (reflected in REM sleep).



89 THE APPLICATION OF PHOTOTHERAPY TO SLEEP DISORDERS IN ELDERLY PATIENTS

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Sleep disorders are frequent in the elderly and most of the complaints concern early morning awakening and poor consolidation of sleep and wakefulness. The results of our preliminary studies have suggested that elderly patients with idiopathic nocturnal sleep disruption (i.e., not secondary to identifiable sleep pathology) have substantially greater reductions of circadian amplitude and/or a more advanced circadian phase than elderly subjects without a sleep disturbance. We hypothesized that phototherapy designed to reverse the abnormalities of circadian organization will lead to improved timing and consolidation of nocturnal sleep in those patients.

Four patients with sleep complaints were studied. Two of them (ages 68 and 73, both female) complained of early morning awakening (before 5 A.M.), and two subjects (ages 72 and 73, both male) were suffering from disrupted sleep. Screening medical and psychological exams were normal. The constant routine protocol was used to estimate the phase and amplitude of the endogenous circadian temperature rhythm in these four subjects, and the timing and duration of the light treatment was designed according to the results of these estimations. After the phototherapy, a second constant routine was conducted to quantify the effects of the treatment on circadian organization. The quality of sleep was assessed by sleep-wake logs and ambulatory activity monitoring during at least a week before and after the treatment. Results showed significant changes in circadian organization and sleep quality after the light treatment and suggest that phototherapy can be successful in the treatment of idiopathic sleep disruptions of the elderly.

90 — withdrawn

91 RAPID ADJUSTMENT TO NIGHT SHIFT USING A SINGLE PULSE OF BRIGHT LIGHT

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Bright light treatment protocols typically involve several hours of illumination on a number of consecutive days. While this time frame is acceptable for many clinical disorders, a more rapid adjustment is required in order to benefit rotating shift workers. In addition, for such interventions to gain acceptance by workers the treatment can be neither too restrictive nor time consuming since the benefits are less tangible and immediate and can conflict with more desirable alternative activities.

Recently, we have shown that when a subject's irradiance is strictly controlled, timed exposure to bright light can produce significant phase shifts. In response to a single 4 hour pulse of bright light between midnight and 4 am. on the first nightshift, 7 subjects working in a "bright light work station" exhibited temperature minima in the second half of the major sleep period (0900 to 1700h) by day 3. In contrast, 7 control subjects not exposed to the single light pulse continued to show minima during the nightshift work period.

The daytime (major) sleep of the bright light group showed fewer arousals, greater sleep efficiency, and more REM sleep in the second half of the sleep period compared to control subjects. Subjective sleep quality was also significantly better in the bright light group and spontaneous termination of sleep occurred less often (i.e., treated subjects typically were still asleep at the designated wake up time (1700)). Also as a consequence of bright light treatment, on-shift sleepiness was reduced and work efficiency and cognitive psycho-motor performance were enhanced on each of the three consecutive nightshifts .

These results indicate that a single 4-hour pulse of bright light during the first night shift may be effective in expediting adaptation to shiftwork both by enhancing daytime sleep and improving on-shift performance. Since treatment occurs on company time and incorporation of the 'workstation' permits the execution of normal duties this intervention shows considerable promise for enhancing adaptation to nightshift in both permanent and rotating shiftworkers.

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BODY TEMPERATURE AND SLEEP/WAKE DISTRIBUTION IN BLIND SUBJECTS WITH AND WITHOUT SLEEP COMPLAINTS UNDER MINIMAL MASKING CONDITIONS

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Blind persons have been the subject of several investigations in order to determine the role of light as a potential zeitgeber. However, most of the studies did not take into account the masking effects of social contact, food intake, body posture and activity. In order to better characterize the circadian output of blind people we have attempted to better control these variables.

Seventeen completely blind male subjects remained resting in bed for 24 hours, receiving an equally distributed diet and sleeping at their convenience. Core body temperature was monitored continuously and the sleep-wake state was assessed behaviorally every hour. According to a sleep questionnaire, subjects were divided into three groups: N=no sleep complaints (n=5), C=chronic complaints in remission (n=5), A=chronic and acute sleep complaints (n=7).

Group N showed significantly earlier sleep onsets of their main sleeping episode than group C ((x+SEM) 22:30 ± 0:41 vs 0:42 ± 0:20; $p<0.05$). This finding corresponds with an earlier temperature minimum in group N (3:34 ± 0:50 vs 9:40 ± 2:19; $p<0.01$). Group A showed no regularity in sleep onset or in the phase of the temperature minimum. They also had a significantly reduced amplitude of their temperature compared to group N (0.32 ± 0.04 vs 0.54 ± 0.06 °C; $p<0.05$)

These findings under minimal masking conditions suggest a higher prevalence of circadian rhythm disorders among totally blind people than previously thought, despite exposure to strong social contacts. The capability of the non-complainers to be entrained to the geophysical day by non light *Zeitgeber* may be related to their early phase position of core body temperature and sleep onset.

93

USE OF POLAR PHASE/AMPLITUDE VECTORS TO EVALUATE THE RESPONSE OF THE HUMAN CIRCADIAN SYSTEM TO LIGHT.

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We have previously reported that a stimulus consisting of three cycles of exposure to bright light (7,000-12,000 lux, 5 hours), ordinary indoor light (150 lux, 11 hours), and darkness (<2 lux, 8 hours) can induce large, rapid shifts of the endogenous circadian pacemaker in human subjects (1). In addition, we found that the stimulus could enhance or diminish the amplitude of the circadian temperature cycle. This implies that evaluation of the effect of light on the human circadian system requires consideration of both amplitude and phase.

In order to do so, we have represented the data from 45 experimental trials reported earlier (1) on polar coordinates, in which the radius indicates the amplitude of the subject's temperature cycle during a constant routine and the polar angle indicates the endogenous circadian temperature phase, forming a phase/amplitude vector (PAV). For each trial, PAV's representing the initial pre-stimulus and final post-stimulus constant routines were plotted in this manner and the vector difference (ΔPAV) was found by joining their endpoints. The resultant ΔPAV is thus a direct measure of the combined change of phase and amplitude induced by the stimulus.

We found that the magnitude of the ΔPAV varied with the initial circadian phase of light administration, reaching a sharp peak near the minimum of the endogenous circadian temperature cycle. The vector length at this phase was approximately 10 times greater than it was at the temperature cycle maximum. These results are consistent with the predictions of Kronauer's mathematical model of the effects of light on the human circadian system (2). According to the model, the ΔPAV represents the combined effects of the drive of the light on the circadian system, amplitude recovery and drift due to circadian period. Further experiments are required in order to determine the relative importance of each of these factors as a function of circadian phase.

(1) Czeisler C.A. et al. (1989) Science 244: 1328-1333.
(2) Kronauer R.E., Czeisler C.A., Brown E.N. (1989) Sleep Res 18: 424.

94 THE PHASE ADVANCING EFFECTS OF MELATONIN ADMINISTRATION IN HUMANS: EVIDENCE FOR A PHASE RESPONSE CURVE. Robert L. Sack, Alfred J. Lewy, Jeanne Latham and Mary Blood. Sleep and Mood Disorders Laboratory, Oregon Health Sciences University, Portland OR, 97210.

Entrainment (by phase advance) of rats free-running in DD occurs if daily injections of melatonin (but not placebo) are given within a narrow time frame of pacemaker sensitivity in late subjective day (maximal effect at CT 10.5; range 9 to 11) (Armstrong SM; *Pineal Research Reviews* 7:157-202, 1989). Melatonin injections did not produce phase delays at any circadian phase. Furthermore, when analyzing the data from our previous studies of melatonin administration to totally blind human subjects with free-running endogenous melatonin rhythms (Sack RL and Lewy AJ; *Sleep Research* 17:396, 1988), we noted maximal phase advances when exogenous administration preceded endogenous melatonin production by about 6 hours. Because not all circadian phases were tested, we cannot be sure that melatonin is without phase-delaying effects in humans.

Based on these observations in animals and blind people suggesting a PRC, we administered melatonin (0.25 mg) in single-blind, placebo-controlled trials to eight normally-sighted male subjects at 1700 and 1900 for four days. Several subjects had additional trials in which melatonin was given up to four hours earlier. Sleep times were held constant. Considering all 14 trials, there was an average phase advance in the endogenous melatonin onset of 34 ± 19 minutes ($p < 0.01$). The magnitude of the phase advance was proportional to the interval between exogenous administration and the onset of endogenous melatonin production for up to six hours ($r = 0.87$; $p < 0.01$) with a fall-off thereafter.

These data suggest that the phase-advancing effects of melatonin in humans are dependent on the timing of admininstration and that maximal phase advances occur when melatonin is given in late subjective day. The full delineation of a PRC awaits further trials. Other factors such as dose, plasma kinetics, the number of daily doses and competition from internal and external zeitgebers may also be important determinants of the phase-advancing effects of melatonin in humans.

95 ENDOGENOUS CIRCADIAN RHYTHM OF THYROID STIMULATING HORMONE CAN BE PHASE SHIFTED BY LIGHT EXPOSURE

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We have recently reported that the daily variation of TSH secretion seen under periodic conditions persists under extended constant routine conditions, reflecting the output of an endogenous oscillatory process (1). In order to better characterize the endogenous control of the TSH rhythm, we have evaluated the endogenous component of the TSH secretory rhythm before and after a light-induced phase shift.

Fourteen normal male subjects underwent a protocol consisting of an initial constant routine (CR) assessment of endogenous circadian phase and amplitude, an intervention regimen which included three consecutive nightly exposures to bright light (5 hrs; ~7-12,000 lux), and a final CR. Blood was sampled every 20 min via an IV catheter during CR's, and TSH was assayed by sensitive IRMA.

Substantial phase shifts of the human circadian pacemaker, as marked by the endogenous components of the core body temperature and cortisol secretory cycles, were seen following three nights of exposure to the bright light stimulus. Preliminary data from the three subjects whose TSH assays have been completed show that the endogenous component of the daily TSH rhythm was shifted with a magnitude and direction comparable to that of the temperature and cortisol cycles.

These data provide preliminary evidence that the endogenous component of the TSH rhythm is coupled to other physiologic rhythms governed by the hypothalamic human circadian pacemaker.

1) Allan JS and Czeisler CA, Submitted, American Federation for Clinical Research, Annual Meeting, Washington, DC, 1990.

96 ACTILLUME ASSESSMENTS OF HUMAN PHOTIC EXPOSURE Daniel F. Kripke, Roger Cole, and William Gruen;* Department of Psychiatry, V-116-A, UCSD, La Jolla, CA 92093. USA and *Ambulatory Monitoring, Inc., 731 Saw Mill River Road, Ardsley, NY 10502.

Because citizens of contemporary cultures spend so much time indoors under artificial illumination, our photic exposures differ greatly in intensity and duration from the outdoor photoperiod. Lewy, Wever, and colleagues have shown that maximal human responses to illumination may be achieved only with illuminations of 2500 lux or more. Therefore, chronobiologically, we spend much of our days in the dark.

The Actillume is a new instrument designed to monitor human illumination exposures in contemporary habitats. Designed to be worn on the wrist, the Actillume also measures accelerations to monitor activity and to infer the sleep/wake cycle. A secondary external phototransducer can be mounted at the eyes, outside of clothing which might cover the wrist, or outside of bedcovers. Nevertheless, simultaneous monitoring of log lux at the forehead and wrist demonstrates a very strong correlation (r=0.93); thus, for many purposes, use of the secondary phototransducer may be unnecessary. Actillume recordings demonstrate that transcontinental travellers who attend scientific meetings may receive insufficient bright light to efficiently overcome jet lag. Some elderly subjects receive scarcely any bright light illumination. Preliminary recordings demonstrate that even experimental phototherapy may produce much less illumination exposure than expected.

97 LIGHT-INDUCED ADAPTATION OF THE HUMAN CIRCADIAN SYSTEM TO NIGHT WORK
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A number of studies indicate that complete physiologic adaptation of the circadian system to night work fails to occur, even among workers on "permanent" night duty schedule. To compare the extent to which scheduled exposure to bright light and darkness could facilitate circadian adaptation to a week of night work, 5 control and 5 treatment studies were carried out in normal young male subjects. During the week of night work, subjects continued to live at home, reporting to the lab each night. To evaluate the extent of physiologic adaptation to the week of night work, the phase of the endogenous circadian temperature minimum (ECP_{min}) was evaluated in conjunction with the 1st and 6th night shifts using a laboratory constant routine. Treatment subjects were exposed to bright light (7-12,000 lux) from 00:15-07:45 on the 2nd through 5th nights of work, while control subjects were exposed to room light (~150 lux) on those nights. After each night shift subjects left the lab and traveled home. Treatment subjects remained in the dark from 09:00-17:00 each day, while control subjects were not scheduled to be in the dark at any particular time.

The average ECP_{min} occurred at 04:59 on the 1st night shift. On the 6th night shift, the average ECP_{min} of subjects in the control studies continued to occur during the night (03:31 $\pm$ 0:56), indicating a lack of adaptation to the night work schedule. In contrast, the ECP_{min} of subjects in the treatment studies shifted an average of 9.6 hours to a significantly later hour (14:53 $\pm$ 0:32, $p < 0.0001$), indicating circadian adaptation to day sleep and night work. Alertness and cognitive performance were also significantly improved in the treatment group during night shift hours.

We conclude that maladaptation to night work can be effectively treated with scheduled exposure to bright light at night and darkness during the day.

98 **LIGHT THERAPY IN SAD PATIENTS: CLINICAL RESULTS AND EFFECTS ON THE INTERNAL PHASE RELATIONSHIP BETWEEN LOCOMOTOR ACTIVITY AND BODY TEMPERATURE RHYTHM**

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The question whether affective diseases are connected with alterations in the circadian system has recently been discussed controversally. In a subgroup of patients who fall ill with depressions every winter (SAD patients; from Seasonal Affective Disorder) Lewy et al. (1987) found a phase delay relation between the melatonin rhythm and the sleep-wake cycle. A treatment with bright white light (2500 lux) in the morning resulted in a re-adjustment of the melatonin rhythm and in an improvement of mood. Hypothesis based on the shape of animal phase response curves are in favour of morning light for the re-adjustment of phase delayed rhythms (Terman et al. 1989).

We treated 33 SAD patients from 6:00h to 8:00h and 18:00h to 20:00h with bright white light (2500 lux) or with dim yellow light (300 lux). After two weeks both groups showed a significant improvement of depression in the estimate of the physician as well as in the self assessment. The group with bright light improved significantly more than the group with dim light (Koehler et al. 1989).

In addition we recorded continuously in nine patients the rhythms of rectal temperature and locomotor activity with a portable data acquisition system before and during the light therapy for about seven days. As a result we could not find an uniform phase angle relation between the two rhythms. In six patients the temperature rhythm was rather phase advanced, in two patients it was phase delayed in relation to the rest/activity rhythm. One patient showed phase instability. The treatment with bright white light corrected the phase position in advanced as well as in delayed temperature rhythms while dim yellow light didn't produce any such effects.

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Lewy,A.J.; Sack,R.L.; Miller,S.; Hoban,T.M. (1987): Antidepressant and circadian phase-shifting effects of light. *Science*, 235:352-354
Terman,M.; Terman,J.S.; Quitkin,F.M.; McGrath,P.J.; Stewart,J.W.; Rafferty,B.(1989): Light therapy for seasonal affective disorder. A review of efficacy. Neuropsychopharmacology, 2:1-21

99 SCN-ABLATION AND HAMSTER HOARDING BEHAVIOR. R. Mistlberger, C.H. Jones. Depts. Psychology, Simon Fraser University, Burnaby, BC and University of British Columbia, Vancouver, BC.

It was previously noted that SCN-ablated hamsters with restricted access to a foraging area may exhibit unusual open field behaviors, including sleeping and moving food from the home cage to the open field (Rusak, Mistlberger, Jones and Losier, 1989). The SCN may participate in setting internal states necessary for ensuring appropriate distribution of behavior in the animal's environment. The present study attempted to replicate these observations in a much larger sample. Male hamsters (16) were trained to hoard food using tunnel-open field boxes as in Rusak et al. Five daily 20 min test sessions were then videotaped and scored. After SCN ablation and a month recovery, the procedures were repeated. There were no qualitative differences in the animals' open field behavior after the lesions; all animals hoarded food back to the home cage and none were ever observed to sleep or move food into the open field. Hoarding was more efficient; latency to collect all food was reduced in all animals. This bore no apparent relation to lesion placement or size (9 total SCN lesions), activity pattern, lighting (LD 14:10 or DDred), or hoard status (daily food supplement or no supplement). The hypothesis that the SCN is necessary for appropriate spatial segregation of species typic behavior is not supported. Supported by NSERC.

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GABAERGIC MODULATION OF 2-DEOXYGLUCOSE UPTAKE IN THE SCN *IN VITRO.*

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Perhaps 50% of the neuronal population of the mammalian suprachiasmatic nucleus (SCN), probable site of the "master" circadian clock, is composed of GABA interneurons. The $GABA_A$ agonist, muscimol, phase-shifts circadian rhythms (Smith &Turek,1986) and light-induced phase delays may be blocked by the $GABA_A$ antagonist, bicuculline (Ralph & Menaker,1989). This study examined whether GABAergic agents could alter the diurnal peak in 2-deoxyglucose (2DG) uptake previously observed *in vitro* (Newman et al.,1986).

SCN slices (500 μ) from male Wistar rats, entrained to LD12:12, were prepared in standard fashion (Newman & Hospod, 1986) around CT2. Experimental slices were pre-incubated in buffer (75 min) followed by buffer plus drug (30 min), whereas control slices were exposed only to the buffer (105 min). Slices were then placed in incubation buffer containing [^{14}C] 2DG, with or without drug, respectively, for 45 min. Slices were then rinsed (30 min) in the presence of drug where appropriate, frozen, sectioned, and autoradiographed. Sections through the SCN were chosen on the basis of Nissl stain and the corresponding autoradiograms were analyzed by an image analysis system. Samples were taken at the dorsomedial and ventrolateral poles of the SCN bilaterally, adjoining anterolateral hypothalamus (AH), and optic chiasm. Relative 2DG uptake (R2DGU) scores from the SCN, normalized to the AH, were averaged across sides and sections to give subject scores.

Analysis of variance revealed that muscimol (10 μMol) greatly attenuated ($p < .001$) SCN R2DGU (dSCN $\bar{x}$ ± S.E. = 1.06±0.05; vSCN $\bar{x}$ ±S.E. = 0.96 ±0.06; n=7) in comparison with controls (dSCN $\bar{x}$ ± S.E. =1.41±0.06; vSCN $\bar{x}$ ± S.E. = 1.27±0.04 ; n=7). In contrast, attempts to reduce GABAergic transmission with the GAD inhibitor, allylglycine (n=7, both groups), or the chloride channel blocker, picrotoxin (n=4, both groups) failed to affect the R2DGU signal in the SCN ($p > .13$). Thus, this measure of diurnal metabolism in the SCN can be reduced by a GABAergic agent (Supported in part by a grant from the Upjohn Company).

101 CONTROL OF THE PERIOD AND PHASE OF CIRCADIAN RHYTHMS RESTORED BY ANATOMICALLY CHARACTERIZED SUPRACHIASMATIC GRAFTS.

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Transplantation to the third ventricle of fetal tissue including the SCN can restore free running locomotor rhythms in adult male hamsters previously subjected to SCN lesions. The period of these rhythms tends to be slightly greater than 24h in DD, and recipients do not entrain to normal intensity LD cycles. In experiment 1, we examined responses to normal (200 lux) and bright (2000 lux) 10L:14D cycles and phase shifts before and after transplantation of SCN or cortical tissue. Experimental hamsters often showed increased masking after grafting. In a few cases, however, free runs began from entrained phase upon release into DD. Phase shifts in response to triazolam were variable. In experiment 2, aftereffects were induced by 12 weeks of exposure to long (14L:12D) or short (14L:9.25D) T cycles for 12 weeks before SCN lesions and transfer to DD. Following SCN transplantation 3 weeks later, the period of rhythms restored to hamsters preexposed to short T tended to be less than that of animals entrained to long T (23.46 ± 0.21h vs. 24.26± 0.28h, n = 12; $p = 0.05$). After rhythmicity was regained, recipients failed to lengthen tau in response to increments in constant light intensity. Some functional grafts were labeled by retrograde transport of fluorescent microspheres injected into the host PVN, PVT, or septum. Rhythmicity was also restored in one instance, however, by a graft ventral to the diagonal band which is unlikely to have formed neural connections with the host. Supported by NSF BNS86-16935.

102 NEURAL TRANSPLANT OF CULTURED SUPRACHIASMATIC NUCLEI. J. Ding, J. Buggy, L. Terracio* and P. J. DeCoursey**. Depts. of Physiology, Anatomy*, School of Medicine, and Dept. of Biological Sciences**, Univ. of South Carolina, Columbia, S.C. 29208.

Our previous fetal SCN transplant study (Neurosci. Abstr. 15: 293.5, 1989) demonstrated that certain peptidergic neurons cluster within the graft into a distinctive multicellular plexus characteristic of the SCN. To further study the functional significance of the clustered aggregation of the graft tissue, we developed a rotary cell culture system feasible for neural transplant after long term culture in vitro. Anterior-basal hypothalamus containing the SCN were dissected, dissociated from perinatal rat and hamster, and incubated in rotary suspension to reform organotypic cell aggregates. Electrophysiological and electron microscopic analyses demonstrated the viability of those cells in rotary culture for up to 4 weeks. Peptidergic neurons characteristic of the SCN (vasopressin, VIP and somatostatin) were immunohistochemically identified in the aggregates indicating that the cells in culture originated from the SCN region. After one week in culture, the aggregates were transplanted into a host's lateral or 3rd ventricle. The aggregates survived and continued to express neuropeptides and cytoskeletal markers for up to 8 weeks after transplantation. Immunofluorescent confocal image analysis examined the reinnervation of NPY terminals from host to graft. However, clustered groupings of these peptidergic neurons were not evident either in the aggregates nor in the transplant analyzed thus far, suggesting that the organization of SCN-like structures may require unspecified growth factors or interaction with a host brain environment at an early stage of embryonic development.

103 SPATIAL DISTRIBUTION AND INTERACTION OF SCN NEUROPEPTIDES IN INTACT HAMSTERS AND RATS AND IN SCN TRANSPLANTS: TOWARDS QUANTITATIVE, 3-DIMENSIONAL VISUALIZATION. J. Buggy and J.D. Ding, Dept. of Physiology, Univ. of SC, School of Medicine, Columbia, SC, P.J. DeCoursey, Dept. of Biological Sciences, Univ. of SC, Columbia, SC 29208.

Characteristic patterns of overlap of the neuropeptides NPY, NP (neurophysin-II) and VIP are regularly seen in immunohistological serial sections of the SCN in normal intact rats and hamsters as well as in transplants of SCN to lateral or 3rd ventricles of SCN-lesioned animals. Computerized image analysis of the central region of intact SCN or the SCN-plexus area of a transplant was utilized to compare these neuropeptide distributions. Videoscans were made of sequential coronal sections of SCN stained immunohistologically for NPY, NP, VIP, and in most cases, SS (somatostatin); the final section of each sequence was stained for Nissl substance. Scans were fiducially aligned, digitized, and stored in memory. A pixel intensity threshold was selected for presence or absence of the neuropeptides in the SCN tissue. The distribution was then quantitatively plotted for each neuropeptide as well as for pairs or triads of the neuropeptides, and the percent overlap determined. Confocal microscopy is also being used to examine intact SCN which have been stained with multiple labels, in an attempt to reconstruct 3-dimensional patterns and detect neuropeptide, synaptic, and cytoskeletal (GFAP and neurofilament) interactions.

VIP and NP in lateral transplants to arrhythmic hosts were clustered as plexi with aggregates of adjacent or overlapping neuropeptides; SS was less clustered but often adjacent to NP. NPY was extensive and diffuse except for a conspicuous absence in VIP and NP aggregates. The composite results suggest that within the transplants of basal hypothalamus, the neuropeptide organization of SCN-like plexi bears a striking resemblance to SCN of intact animals.

104 **LOCALIZATION OF A CIRCADIAN PACEMAKER TO THE VENTROLATERAL SUPRACHIASMATIC NUCLEUS (SCN).** **M.U. Gillette and T.K. Tcheng. Dept. of Physiol. & Biophys. and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.**

The mammalian SCN contain an endogenous circadian pacemaker. Little is known of the pacemaker's intrinsic organization. Persistence of behavioral circadian rhythms after partial SCN lesions demonstrates that this structure need not be intact to drive circadian rhythms. The question arises, "Is the pacemaking function restricted to a particular region in the SCN, or is it distributed?" Anatomical and immunohistochemical studies of the SCN have revealed striking differences between neurons in the dorsomedial (DM) and ventrolateral(VL) SCN (van den Pol 1980, 1985). We hypothesize that the pacemaker is localized in one of these two regions.

In order to test this hypothesis, we prepared SCN in hypothalamic brain slices, surgically isolated progressively smaller regions of the SCN by microdissection, then examined ensemble neuronal activity for circadian rhythms (CRs) *in vitro*. Our previous work has shown that a 500 μm coronal slice from 2-5 mo Long-Evans rats, which contains less than the anterior-posterior extent of the SCN, produces a stable CR for at least 3 days *in vitro*. Trimming the slice to within 100 μm of the paired SCN results in an unperturbed CR. Neuronal activity in both the DM and VL regions in the intact slice peaks synchronously at CT 6.9 on day 2 (N=8).

Our current research further localizes the pacemaker. Bisecting the SCN by severing the commissure connecting the two nuclei has no apparent effect on the CR (N=4). Subdividing the bisected SCN into DM and VL halves results in a marked difference in CRs in these two regions. The VL region exhibits a peak in neuronal activity near CT 6.9 on day 2 (N=8). The DM-SCN does not exhibit a noticable peak in activity (N=6). These results support the hypothesis that the circadian pacemaker is localized, not distributed. Furthermore, they demonstrate that the VL-SCN contains a circadian pacemaker. Whether the DM-SCN contains a pacemaker whose electrical CR is uncoupled by the surgery remains to be determined.

105 PROPERTIES OF CULTURED SUPRACHIASMATIC NUCLEUS CELLS.
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Cell cultures of neurones are a useful tool in neurosciences for a variety of reasons. Some investigators are studying the effect of transplantation of cell suspensions or cells from cultures, whereas others use in vitro systems to study the uptake of neurotransmitter substances.
In order to study electrophysiological properties of SCN cells with the patch clamp technique, SCN cells from several rat fetuses aged 21-22 days were cultured in a chemical defined medium. Addition of nerve growth factor to this medium, while omitting fetal calf serum not only increased survival of the cells, but also blocked the glial overgrowth very efficiently. The mechanically dissociated SCN cells start to reaggregate between day 2 till day 5 producing interconnected reaggregates. There are about seven interconnected bundles per aggregate with about 10 million dissociated cells per ml. Not only inter but also intra aggregate bundles are present. From a rough estimation (within aggregates counting is nearly impossible) the amount of bipolar cells is 60%, while 40% belongs to the multipolar type. Immunocytochemistry with monoclonal antibodies against neurofilament demonstrated that these cells are neurones. (The neurofilament antibody detects the 50, 70, 150 and 200 KD phosphorylated neurofilament.) Lucifer yellow liposome uptake affirmed these results (for details of the technique see Neuroendocrinology 48: 445-452, 1988). Within aggregates VIP (UCB, Bioproducts, Bruxelles) could be demonstrated within the SCN neurones, while after 24 hour's incubation with the primary polyclonal antibody light microscopically VIP was found within granules too. Vasopressin (Immuno Nuclear Co, Stillwater USA) was located within the neurones and their protrusions into the inter-aggregate connections. From day 7 our bipolar neurons showed sponteneous action potentials with a skewed interval distribution and a mean firing frequency less than 0.5 Hz. In response to depolarizing currents the firing was regular with a maximum frequency of 30 Hz. Under voltage clamp these neurons exhibited inward Na-currents (TTX sensitive) and pronounced outward currents, presumably carried by K- and Cl-ions.

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THE PIGEON SUPRACHIASMATIC NUCLEUS (SCN) AND INTERGENICULATE LEAFLET (IGL): A TRACT TRACING AND IMMUNOCYTOCHEMICAL STUDY. R. B. Norgren, Jr. and Michael N. Lehman, Dept. Anat. & Cell Biol., Univ. of Cincinnati Coll. Med., Cincinnati, OH.

The precise location of the avian SCN has been the subject of much controversy. Two nuclei in the bird hypothalamus, the medial hypothalamic nucleus (MHN) and the lateral hypothalamic retinorecipient nucleus (LHRN), have been proposed as homologs to the mammalian SCN. We have examined this issue in the pigeon with a variety of markers that delineate the SCN in mammals. Intravitreal injections of rhodamine isothiocyanate revealed an arch of labeled retinal axons in the lateral hypothalamus. A terminal field in this nucleus was observed after intraocular injection of WGA-HRP. No evidence of retinal input to the medial nucleus was observed. Both the MHN and LHRH were examined with a variety of antibodies to peptides found in the mammalian SCN: vasoactive intestinal polypeptide (VIP), neurophysin (NP), and neuropeptide Y (NPY). Since the hamster SCN also contains cholecystokinin (CCK) neurons, this peptide was also examined. VIP and magnocellular NP neurons were found in close proximity to both the MHN and LHRN. Neither MHN nor LHRN contained notably dense fiber staining with any of antibodies tested. There was sparse NPY-like staining in both the MHN and LHRN. In the thalamus, a cluster of NPY-like immunoreactive neurons was found in close proximity to the nucleus rotundus. Double-label studies indicate that retinal fibers lie in close proximity to these neurons. As these are the only NPY-like immunoreactive neurons in the pigeon diencephalon, they may constitute a cell group homologous to the mammalian IGL. [Supported by NIH NS28175 to M.N.L.]

107 THE STATISTICAL ANALYSIS OF CIRCADIAN PHASE AND AMPLITUDE IN CONSTANT ROUTINE CORE TEMPERATURE DATA

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Accurate estimation of the phases and amplitude of the endogenous circadian pacemaker from constant routine core temperature series is crucial for making inferences about the properties of the human biological clock from data collected under this protocol. This talk presents a set of statistical methods based on a harmonic regression plus correlated noise model for estimating phase and amplitude of the endogenous circadian pacemaker from constant routine core temperature data. The methods include a Bayesian Monte Carlo procedure for computing the uncertainty in these circadian functions. We illustrate the techniques with a detailed study of a single subject's core temperature series and describe their relationship to other statistical methods for circadian data analysis.

108 ROLES OF SLEEP AND CIRCADIAN RHYTHMICITY IN MODULATING PITUITARY-DEPENDENT SECRETIONS AND GLUCOSE REGULATION IN MAN. J. D. Blackman, D. Roland, J. Sturis, T. Marcinkowski, K. S. Polonsky, E. Van Cauter, Department of Medicine, University of Chicago, Illinois 60637.

In normal man, secretion of cortisol (F), TSH and GH are profoundly modulated by diurnal variations. Our aims were to delineate the relative roles played by sleep and circadian rhythmicity in causing these diurnal variations and to determine whether glucose regulation, which may be influenced by both GH and F, is also modulated by sleep and time of day. Eight normal young men, ages 22-27 yrs, were studied over a 53-h period including sleep during normal bedtime hours (23-07), a complete 24-h cycle with total sleep deprivation and an 8-h daytime period of recovery sleep (11-19). All studies were preceded by two nights of habituation. To provide a constant level of stimulation of pancreatic function, glucose was infused at a rate of 5 g/kg/24 hrs for 57 hrs and oral feeding was withheld. Sleep was monitored. Blood samples for the measurement of TSH, GH, F, glucose (G) and C-peptide (CP) were drawn at 20-min intervals throughout the study. In the evening preceding normal nocturnal sleep, TSH, CP and G started rising and continued to rise after sleep onset to reach a maximum around midsleep. These nocturnal G, CP and TSH maxima represented increments above daytime levels of 31± 15%, 43 ± 18% and 77 ± 29 %, respectively . A GH pulse occurred within 20 min after sleep onset in all subjects. F declined throughout the evening, reaching minimum values of 1.7 ± 0.3 µg/dl during the first 2 hrs of sleep (23-01). In the evening preceding the night of sleep deprivation, levels of G and CP increased again. G and CP reached a maximum around the usual bedtime (corresponding to increments of 15 ± 13% and 39 ± 15 %, respectively) but then started declining. In contrast, the rise of TSH was greatly enhanced and slightly prolonged in the absence of sleep (corresponding to an increment of 143 ± 87%, $p<0.01$). F levels during the period 23-01 were higher than in the presence of sleep (3.3 ± 0.5 µg/dl, $p<0.05$) . GH secretion during sleep deprivation was strongly decreased but still detectable in 5 of the 8 subjects. Following onset of daytime sleep, G and CP rose abruptly to reach a maximum within the first 3 hrs of sleep (average relative increment 16 ± 10% for G and 46 ± 16 % for CP). During daytime sleep, TSH levels were similar to those observed during wakefulness. A GH pulse of magnitude similar to that observed during nocturnal sleep, occurred during daytime sleep in all subjects. F levels were approximately 25% lower during the first 2 hrs of daytime sleep (11-13) than on the previous day at the same time. We conclude that pituitary-dependent secretions and glucose regulation are markedly modulated by both time of day and sleep.

109 CIRCADIAN TIMING OF SLEEP IN LONGHAUL FLIGHT CREWS. P.H. Gander and R.C. Graeber, Aerospace Human Factors Research Division, NASA Ames Research Center, Moffett Field, CA 94035.

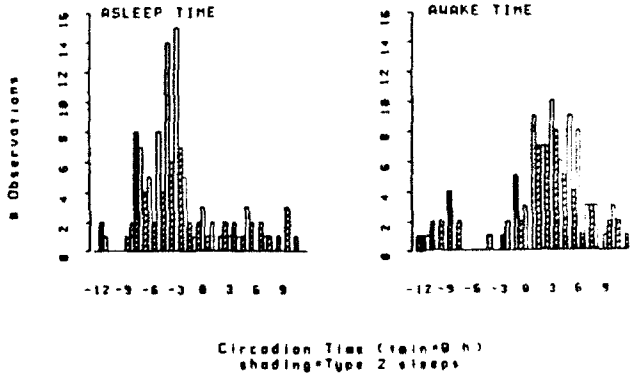
Twenty-nine male flight crew members (mean age 52 years) were monitored flying one of four commercial longhaul trip patterns including either multiple Atlantic crossings (+/- 8 h or +/- 10.5 h), multiple Pacific crossings (+/- 7 h), or long north-south Pacific flights (+/- 2 h). The average layover duration of 24.75 h permitted selection of sleep times across a broad range of circadian phases. Rectal temperature was monitored continuously (2 min samples, Vitalog PMS-8). Sleep and nap times were noted on awakening. Complex demodulation was used to locate temperature minima. The period of the temperature rhythm was determined by linear-non-linear least squares iterative multiple regression. Significant differences are from 2-way ANOVA (cycle type by sleep type).

First sleeps (Type 1) in a layover followed significantly longer wake periods and were rated as significantly better than subsequent (Type 2) sleeps, although their durations were not significantly different. The peak of the circadian asleep distribution, particularly for Type 1 sleeps, is slightly earlier than in desynchronized free-run (Strogatz, 1986) but clearly later than in entrainment. Wakeup normally occurs on the rising phase of the temperature rhythm with the notable exception of 11 Type 2 sleeps with wakeups just after the temperature maximum. These were very short "anticipatory sleeps" attempted after short wake durations at non-optimal circadian phases just before going on duty.

Wake durations were calculated from sleep/nap wakeup to sleep/nap onset (s/n cycles) and from sleep wakeup to sleep onset (s/s cycles). Wake/sleep patterns were complex combinations of long (Type 1) cycles (mean s/n 22.5 h, s/s 24.6 h) and short (Type 2) cycles (mean s/n 14.2 h, mean s/s 17.4 h). Nevertheless, 82% of the subjects had significant temperature rhythm periods in the range 24-27 h (mean 25.7 h).

These data indicate that the circadian system is an important regulator of sleep timing under extremely complex operational conditions. Faced with rapid sequences of time zone shifts well beyond its entrainment capacity, the circadian system appears to adopt an average period somewhat longer than in free-run.

Strogatz, S.H. (1986) *The Mathematical Structure of the Sleep-Wake Cycle.* Springer-Verlag, Berlin Heidelberg.



110 TRIAZOLAM-INDUCED PROLACTIN (PRL) AND GROWTH HORMONE (GH) RELEASE IN NORMAL MEN SUBMITTED TO AN 8-HOUR DELAY OF THE SLEEP-WAKE CYCLE.

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The aim of the study was to investigate whether triazolam administration following an abrupt shift of the sleep-wake cycle would alter the secretion of PRL and GH, i.e. sleep-dependent hormones. After 3 nights of habituation, 6 normal males, 21-30 y.o., were studied once with placebo and once with triazolam, in random order, at 2-month interval. In each study, the 24-h profiles of plasma PRL and GH were obtained at 20-min intervals, together with polygraphic sleep recordings, under basal conditions, and 1, 3 and 5 days after an 8-h delay of the sleep-wake cycle obtained by sleep deprivation from 23.00 to 07.00 on day 1. A bedtime schedule of 07.00 to 15.00 was enforced for 5 consecutive 24-h periods. Meals were served at 16.00, 20.30 and 03.00, naps and recumbency were not allowed from 15.00 to 07.00. Triazolam (0.5 mg) or placebo was given at 04.00 on the first shifted night and at 07.00 on the following nights. Baseline sleep, PRL and GH profiles were normal. On days 1 and 3 after the shift, the sleep structure was disrupted under placebo (REM distribution was skewed to the early part of the night) but remained normal under triazolam. No significant differences were observed on day 5. On day 1, significant elevations of PRL and GH levels occurred between 04.00 and 07.00 (i.e. between drug administration and sleep onset) after triazolam but not after placebo, and the 24-h GH secretion was higher under triazolam. On day 3 (when the drug was given at bedtime), PRL levels during the sleep period and the magnitude of the sleep onset GH pulse were higher under triazolam than under placebo, resulting for both hormones in elevated 24-h mean concentrations. On day 5, PRL levels were similar under both treatment conditions, but the sleep onset GH pulse was larger (and occurred earlier) after triazolam. On all occasions, the triazolam-induced hormonal elevations were relatively modest, resulting in plasma levels well within the normal range. These results show that following an 8-h delay in the sleep-wake cycle obtained by sleep deprivation, PRL and GH secretions are stimulated by triazolam. These effects are not mediated by sleep, since they also occur during wakefulness. The temporal organisation of GH and PRL secretion is not altered by triazolam.

111 EFFECTS OF THE "JET LAG DIET" ON THE ADJUSTMENT TO A PHASE ADVANCE

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It is widely assumed that the unpleasant symptoms (insomnia, fatigue, malaise and others) associated with jet lag and shift work are due to abnormal phase relationships among biological rhythms. A countermeasure that would hasten the normalization of phase relationships would be extremely useful. One of the most widely promoted countermeasures is the so-called "jet lag diet" [1]. This countermeasure system involves alternating days of feasting and calorie restriction, timed consumption of methyl xanthines, high protein breakfasts and lunches, high carbohydrate dinners, careful exposure to light and an exercise program.

We recently tested the diet plan using our established jet lag simulation [2]. Fifteen healthy, normal men (ages 18-25) were isolated from all temporal cues for 15 days. Seven subjects were controls (no countermeasure), and 8 were the diet countermeasure group. For the first 7 days, subjects slept according to their usual schedules. During the 7th night, subjects were awakened 6 hours early. On the following evening, they retired 6 hours earlier. The phase advanced schedule was maintained for the remaining 8 days of the study. During the entire study for each group, sleep and wake periods, meals, exercise and showers were scheduled, and the same time intervals between events were maintained after the phase advance. According to the jet lag diet, subjects began alternate days of feasting and calorie restriction for 3 days before the scheduled phase advance. Food choices were limited to ensure that high protein breakfasts and lunches and high carbohydrate dinners were selected. Control subjects were able to eat their usual diet. At dinner on the evening before the shift, subjects consumed 1-2 cups of coffee or tea. The day after the shift was also a feasting day. Subjects in the diet group were able to restructure their macronutrient composition as required, calories on the restricted day averaged 1032 Kcal. Data collected included rectal temperature, polysomnography, subjective alertness, mood ratings, and cognitive and motor performance tasks.

The control and diet groups did not differ before the shift in minutes of sleep, percentages of sleep stages, sleep efficiency, sleep latency or REM latency. During the abbreviated sleep episode, the groups were found to differ in total sleep time and in sleep efficiency. Subjects in the diet group slept an average of 30 minutes less than control subjects and were 31% less efficient as well. The decrease in sleep time and efficiency was due to an increase in sleep latency in the diet group (11.4 min to 45.2 min). The latency to slow wave sleep increased by 32%, and the percent of SWS was also 28.5% lower in the diet group only on that night. Aside from the negative effects on sleep on the night of the phase advance which we attribute to the caffeine consumption at dinner, the diet did not alter the rate of adjustment of the core temperature rhythm, or improve mood or performance after the shift above that of the control subjects. These data suggest that the "jet lag diet" is not effective in overcoming jet lag in young subjects.

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112 HORMONAL RHYTHM IN DEPRESSED PATIENTS.

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The hormonal 24-h profiles of 11 normal young volunteers were observed under standard nycthemeral conditions and under a constant routine protocol consisting of a 26-h constant conditions (constant light, temperature, bi-hourly snacks, continuous bedrest, sleep deprivation). The nadir of the plasma cortisol rhythm was slightly advanced and the amplitude of the melatonin, prolactin growth hormone and body temperature rhythms was reduced under constant environment when compared to nycthemeral conditions. In contrast, the nocturnal rise of plasma TSH was slightly higher under constant then under nycthemeral conditions. Our findings point out some similarities between the 24-h hormonal pattern of normal subjects living under constant environment and those of depressed patients living in a natural environment. This observation further supports the hypothesis that an impairment of the entrainment processes of internal biological clocks by environmental time cues may be involved in the pathogenesis of depressive illness.

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SHORTER SUBJECTIVE SLEEP OF HIGH SCHOOL STUDENTS FROM EARLY COMPARED TO LATE STARTING SCHOOLS . Richard Allen and Jerry Mirabell
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Sleep phase delay and problems with morning sleepiness have been reported to occur for adolescents and may represent a significant problem for this age group. To evaluate this a survey questionnaire was developed to cover sleep and waking habits for Tuesday through Thursday and for Friday and Saturday. Test-retest reliability for questionnaire items exceeded 0.80. This survey was then given and returned by all of the students available in a 1st period class in two suburban high schools from different counties. The starting time for the first school was at 8:00 am compared to 7:30 am for the second school.

Sixty-one students were surveyed (21 from school 1 and 40 from school 2); 57% were males and the mean age was 17.13 years. The two groups were almost identical for age and sex. Both samples report for week days remarkably short sleep times (about 7 hours) with bed times close to 11pm. On weekends, with few constraints on their sleep-wake schedule, these adolescents report bed times around 1am with long sleep times of nearly 9 hours. The preferred bedtime on weekends is very delayed (2am or later) for 20% of the students. Comparing the two groups shows, as expected, the significantly later wake time for the school starting later, but the bed times for the two schools did not differ significantly. The number of students reporting short sleep (6 hours or less) was greater for the school starting earlier (15% vs. 0%, $p < 0.05$)) with more daytime sleepiness for this group. These data are consistent with the hypothesis that starting school earlier reduces time in bed and increases the number of adolescents with short sleep times. This age group may have trouble adjusting to earlier wake times.

Overall, timing of the school day does not seem reasonable given the adolescent's circadian rhythm. For the day time they reported on average least alertness at about 10:00am; 64% report they were least alert at 8 or 9am. In contrast 50% of the students reported being most alert after 3pm. about when they were released from school .This may reflect the boredom of school or the tendency toward a late sleep phase, as also suggested by the weekend sleep habits. and the shorter sleep with earlier school time.

114 Treatment of Persistent Sleep-Wake Schedule Disorders with Metyl-Cobalamin (Vitamin B12)

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Only a few reports have discussed the Vitamin B12 treatment of persistent sleep-wake schedule disorders such as the delayed sleep phase syndrome (DSPS) and the non-24-hour sleep-wake syndrome (hypernychthemeral synd.). We successfully treated two adolescent patients suffering from these sleep-wake schedule disorders who complained of not being able to attend school.

A 15-year-old junior high school girl with DSPS was treated with the administration of Vitamin B12 (mecobalamin) 3mg daily. Her sleep onset time was about 02:00 and sleep offset time was around noon. Under the administration of mecobalamin, her sleep period time (SPT) was gradually decreased from 10 hours toward 7 hours with phase-advance shift. She was able to wake up by 07:30 after one-month's administration of VB12.

A 17-year-old high school boy with hypernychthemeral syndrome was also treated with Vitamin B12. His τ before administration of the drug was 24.6 hours and it was reset to 24.02 hours after treatment. The patient's body temperature cycle, also, became more regular and more distinct than before treatment. Under the administration of mecobalamin, these two patients were finally able to return to their classes after a 6-month or more absence.

The mechanism of the efficacy of metyl-cobalamin on persistent sleep-wake schedule disorders is still unknown. Studies on the effects of methy-cobalamin on the sleep-wake rhythm of humans should be encouraged from two metabolic aspects. One is from the aspect of transmethylation including melatonin metabolism thought to affect photoperiodicity. The other is from that of the cholinergic motabolites including cholinomimetic agents such as lecithin thought to shorten the first REM latency and the first interval of REM sleep.

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NON-PHOTIC PHASE RESPONSE CURVES IN WILD TYPE AND tau MUTANT HAMSTERS.

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Non-photic events (novelty-induced wheel running in DD) can produce phase shifts of hamsters' circadian rhythms that are of similar magnitude to shifts produced by light pulses (ca. 3 h advances). Possibly the same constraints limit the maximum phase shift obtainable, whether this is initiated by photic or by non-photic pathways. The period mutation, tau, in golden hamsters is accompanied by an increased response to light pulses. This might result from some peripheral change or from a central change in the pacemaker. Since photic and non-photic responses involve different pathways to the pacemaker, we asked whether responses to non-photic stimuli were also altered in tau mutants. We found that non-photically induced phase shifts in these mutants can be much larger than those in wild type hamsters, and that the shape of the non-photic phase response curve was altered. The tau mutation evidently affects much more than free-running periodicity.

116 **QUANTITATIVE ASSESSMENT OF EXERCISE DEPENDENT ENTRAINMENT IN THE MOUSE**. Dale M. Edgar, Connie E. Martin and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA.

The process of circadian rhythm entrainment has long been thought to depend exclusively on periodic cues in the external environment. However, recent evidence that forced activity and animal handling can phase shift circadian rhythms has raised the possibility that cues arising from spontaneous physiological and/or behavioral events may feedback to the biological clock. To investigate this question we afforded mice scheduled opportunities to exercise in a running wheel under otherwise free-running conditions while monitoring their sleep-wake and drinking patterns, and assessing the amount of spontaneous exercise performed.

Fifteen male mice (Mus musculus, C57BL/6NNia, age 6-12 mos) were surgically prepared for chronic EEG and EMG recording, and individually housed in cages equipped with a swivel commutator and a running wheel. Arousal state, drinking and wheel running were monitored using SCORE, an automated sleep scoring and data collection system. Mice were maintained in constant dark (DD) and food and water were available ad libitum throughout the study. Wheel running opportunities were controlled by restricting wheel rotation to scheduled times each day via a computer-controlled solenoid. Mice were permitted to free-run in DD for 6-8 weeks, after which they were afforded a series of 12-hr, 6-hr, 3-hr and 1-hr windows of daily (T = 24) wheel availability commencing at the same clock time each day. Each wheel availability protocol was maintained for a minimum of 6 weeks.

When running wheels were chronically available, all animals exhibited free-running circadian rhythms with periods significantly less than 24 hrs ($p < .01$). Under all conditions of running wheel availability, mice ran spontaneously and vigorously. 12-hr and 6-hr exercise windows entrained all but 4 mice, with steady state entrainment achieved only when exercise commenced during the latter half of the animals normal circadian waking/active period (CT 18-20). Entrainment was achieved by phase delays of the circadian clock. The remaining animals exhibited relative co-ordination of sleep-wake and drinking circadian rhythms, with phase advances and phase delays occurring depending upon the circadian phase at exercise onset. Surprisingly, all animals entrained to the 3-hr exercise windows and remained entrained when the exercise windows were shortened to 1 hr. Net exercise (indexed by the total wheel running distance) during 6-hr exercise windows (2.51 ±0.16 km) was greater than that during 3-hr (2.06 ± 0.15 km; $p < .05$) and 1 hr windows (0.72 ±0.11 km; $p < .01$). However, the peak running rate (exercise intensity) was essentially the same during the first hour of running (13 meters/min) in each paradigm.

These results demonstrate that spontaneous daily exercise provides feedback to the mouse circadian clock. In addition, it appears that timing and the intensity of exercise are the key determinants of this entrainment process.

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117 CYCLOHEXIMIDE BLOCK OF LIGHT-INDUCED PHASE SHIFTS IN THE ACTIVITY RHYTHM OF THE HAMSTER. Alfred B. Lord, Joseph S. Takahashi and Fred W. Turek. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208

The ability of light to phase shift the circadian clock is the basis of entrainment to environmental light cycles. Although the physiological mechanisms that underlie this process are not understood, work with invertebrate systems has shown that protein synthesis is required for phase shifts to occur. We have investigated the effects of the translational protein synthesis inhibitor, cycloheximide (CHX), on light-induced phase advances in a mammalian system. Syrian hamsters (N = 18) were allowed to free-run in constant dark conditions. Activity onset was designated as circadian time (CT) 12. The animals were given CHX (65mg/kg) at CT 18.5, followed by a light pulse at CT 19. Two control treatments were used to determine the phase shifting effects of a vehicle injection, given at CT 18.5, followed by a light pulse at CT 19, and the CHX alone, given at CT 18.5. The intensity and duration of the light were adjusted to yield 70-80% of the maximum possible phase shifts at this CT. The animals were separated into three groups, each of which received all three treatments in a different order. Hamsters that received injections of vehicle followed by a light pulse showed the expected phase advances of over 100 minutes, significantly different from those that received injections of CHX followed by a light pulse (mean = 35 minute delay) and those that received CHX alone (mean = 55 minute delay). Phase shifts of hamsters that received CHX and light were not significantly different from those that received CHX alone. Two-way ANOVA showed no significant effect of treatment order on phase shifting among the hamsters. These results indicate that protein synthesis is involved in the process of light-induced phase-shifting of the mammalian circadian clock.

118 EFFECTS OF CONTINUOUS ILLUMINATION ON THE SENSITIVITY OF THE HAMSTER CIRCADIAN PACEMAKER TO BRIEF LIGHT PULSES. Dwight E. Nelson and Joseph S. Takahashi Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208.

The circadian system of the hamster has become a model for the study of photic inputs to mammalian circadian pacemakers. Previous studies have shown that, unlike other visual pathways, this system does not adapt to light stimulation of 300 s in duration. We have now measured the responsiveness of this system to brief light pulses delivered in constant light. Golden hamsters were placed in constant light (530 nm; HW = 45 nm) of 1.7×10^{6} to 2.5×10^{14} photons cm^{-2} s^{-1}. A "saturating" light pulse (2.5×10^{14} photons cm^{-2} s^{-1}; 300 s; 503 nm) was delivered to each hamster at circadian time 19 after 1 and 6 weeks of constant conditions to measure the responsiveness of the hamster phase-shifting mechanism to light pulses. The magnitude of the steady-state phase shifts of the running-wheel activity rhythm was estimated after each stimulus. Phase advances induced by the brief light pulses were reduced in magnitude for animals in the higher levels of constant light. In backgrounds of less than 2.9×10^{11} photons cm^{-2} s^{-1} the phase shifts after 1 week were similar to those induced in animals left in darkness. For animals in higher levels of constant light the phase advances were significantly smaller in magnitude. Phase shifts could not be induced in animals in the highest irradiance backgrounds. The decreasing responses with increasing background irradiance were fit by a Naka-Rushton function and the half-saturation constant for the fit was 3.9×10^{10} photons cm^{2} s^{1}. This value is not significantly different from the half-saturation constant for the function describing the sensitivity of this system to light *pulses* delivered in constant darkness. These results are consistent with the hypothesis that the sensitivity of the hamster circadian system to light is not changed by light adaptation during constant illumination. Instead, the continuous background illumination appears to saturate the photoreceptive pathway that mediates the effects of brief light pulses to the hamster circadian pacemaker.

119 RED LIGHT PULSES CAUSE PERIOD AFTER-EFFECTS IN THE CLOCK OF THE UNICELLULAR DINOFLAGELLATE *GONYAULAX*

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In higher organisms, effects on free running period (tau) subsequent to phase shifting light pulses have been reported in several species, and interpreted by some authors as being a result of interaction between different oscillators (Pittendrigh and Daan; Boulos and Rusak). In unicellular organisms, including *Gonyaulax*, such effects have not been reported after pulses of white light that cause phase shifts. Also, with blue light pulses given on an otherwise constant red background, circadian periods of the glow rhythm following the pulse are unchanged or only slightly shortened.

However, when cells kept in constant dim blue light are interrupted by exposures to pulses of red light, persistent period changes (increases) of up to one hour occur. Such after effects are equally strong at all phases of the circadian cycle, and are thus independent of the red light- PRC,thus whether the pulse caused an advance, delay or no phase shift. Measurements subsequent to the pulses have been continued for only a week, thus making it difficult to distinguish between true after-effects and transients. However, transients are generally identified by the fact that day to day changes in tau occur until the period reaches a stable state. The post-pulse periods measured in our experiments can be described as stable on the basis of the linear regression through the daily glow peak, indicative of an after-effect.

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MELATONIN GUIDES A SENSITIVE PERIOD IN BIRDS.

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During times of migratory restlessness migrants *(Pied Flycatchers)* at the age of 8 weeks cannot orient following removal of the pineal gland, whereas the controls and shams show the species specific south-west direction when tested in funnels. Pinealectomy performed at the age of 12 weeks does not influence the orientation capability of the birds.

Melatonin injected in the evening into the breast muscle of the younger birds restores the orientation completely.

It is concluded that melatonin influences the timing of the sensitive period in which the inborn information about migratory direction is expressed from the genes. After measuring the local earth magnetic field this information may be transferred to the memory.

Thus, in general, melatonin may play an important role in the timing of imprinting processes.

121 **ACUTE INFLUENCE OF MELATONIN ON THE CIRCADIAN MELATONIN RHYTHM.**
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Melatonin can entrain activity rhythms and may influence a variety of other circadian rhythms. Since the pineal production of melatonin is itself driven by the circadian system, the present study tested the hypothesis that melatonin acutely affects the circadian melatonin rhythm in the adult Djungarian hamster. Adult hamsters were reared in 16L:8D (lights on 0300-1900 h) and were injected on day 1 of the study with melatonin (5 μg/0.2 ml saline, s.c.) at 0900 h (n = 80) or 1600 h (n = 74). Animals were kept in constant dark on day 2 except for a dim red light. Over the two-day study, animals (n = 3-5) were killed at selected clock times. On day 2, irrespective of the time of day of injection, melatonin content in the pineal or concentration in serum increased within 0.5 h of subjective lights off to a peak 5-7 h later, and remained elevated for up to 10 h. The rise, fall, peak time, peak amplitude, and duration of increased melatonin in the pineal and serum was also the same compared with the circadian pattern of melatonin in untreated controls (n = 76; $p > 0.05$, ANOVA). Melatonin during the subjective day averaged 0.05 ng/gland and 15 pg/ml, while during subjective night increases were as much as 100-fold in the pineal and up to 5-fold in serum. Since a brief exposure to light at night can phase shift subsequent pineal melatonin rhythms, two additional groups of hamsters were exposed on day 1 to 5 min of light beginning at 2300 h (n = 64 each); one group was administered melatonin (5 μg) on day 1 at 2245h to block the light-induced reduction in circulating melatonin. The light pulse delayed the rise, as well as shortened the duration of increased melatonin (1-3h) in both the pineal gland and serum during the subjective night of day 2 whether or not hamsters were treated with melatonin on day 1. Within each group, a temporal correlation was evident between serum and pineal melatonin. The data suggest that melatonin injections are unable to block the effects of light or acutely influence the circadian melatonin rhythm in the pineal gland and in circulation. The mechanism whereby melatonin affects circadian rhythms may be indirect or require more chronic treatment with melatonin. (Supported by NIH HD 22479)

122 SPECIFICITY OF CIRCADIAN RHYTHMS IN DIFFERENT INBRED STRAINS OF LABORATORY RATS.
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Genetic differences between inbred strains have only recently been utilized to investigate the genetic basis of biological rhythms in mammals such as mice, golden hamsters, and rats. In the present study, wheel-running activity was recorded in 3 inbred strains of laboratory rats (ACI/Ztm, BH/Ztm, LEW/Ztm) under light-dark entrainment (LD 12:12) and constant dark conditions (DD).

Significant strain differences were observed in the daily pattern of activity, the amount and duration of activity, the phase angle of entrainment to LD 12:12, and the free-running period under DD. Strain ACI exhibited a unimodal circadian activity pattern as it is commonly described for laboratory rats. Strain BH, however, showed a bimodal activity pattern with two activity bouts about 12 h apart and strain LEW showed an activity pattern with 2-3 activity bouts about 4-5 h apart. Significant strain differences were found in the time of activity onset and offset relative to the light-dark cycle, with BH < LEW representing "early" strains and ACI representing a "late" strain. Significant differences were also observed in the overall level of activity (ACI > BH > LEW) and in the amount of time spend in the wheel (ACI = BH > LEW), indicating genetic influences on both parameters. All these characteristic patterns persisted under constant dark conditions, demonstrating their endogenous character. Power spectrum and periodogram analysis revealed significant differences in the free-running circadian period as well as in the appearance of additional ultradian components for the BH (12 and 6 h) and LEW (4.8 and 4 h) strains.

The results indicate that strains BH and LEW are characterized by a distinct coupling of circadian oscillators. Such strains should prove valuable in subsequent genetic, physiological, and anatomical investigations of the multi-oscillatory nature of the vertebrate circadian system.

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123 PHOTOPERIODIC TIME MEASUREMENT IN GOLDEN HAMSTERS: A TEST OF THE DURATION HYPOTHESIS.
M. Watson-Whitmyre, C. Rogers, L. Nicholson, M.D. Rollag[1] and M.H. Stetson. School of Life & Health Sciences, University of Delaware, Newark, DE, 19716 and [1]Department of Anatomy, USUHS, Bethesda, MD, 20814.

What is the critical parameter of the pineal melatonin rhythm that provides photoperiodic information to the reproductive system? Experiments using Djungarian hamsters and sheep have led many to conclude that the duration of nightly melatonin production is the critical signal. To test this hypothesis in golden hamsters, we injected groups of animals housed on short days (LD 12:12) with propranolol, which blocks melatonin production. At the dosage used, propranolol given during the dark period curtailed pineal melatonin production within 15 minutes of injection; melatonin remained undetectable for the remainder of the night. Injections were given daily for 10 weeks - long enough to assess the gonadal response. As expected, non-injected control animals exhibited gonadal regression. Gonadal regression was prevented in hamsters that received propranol 6 to 10 hours after lights off. However, propranolol injected 4 or 5 hours after lights off did NOT interfere with testicular regression, even though melatonin production in these animals was limited to a low amplitude peak of only 2 hours duration. These results cannot be explained by assuming that propranolol may have pharmacological effects that interfere with normal photoperiodic responses. We have found that propranolol itself does not *cause* gonadal regression when injected into pinealectomized hamsters nor does it *prevent* regression in intact hamsters that receive concurrent melatonin injections. In addition, propranolol does not appear to affect entrainment to the light cycle, as assessed by locomotor activity. We conclude that neither the duration nor the amplitude of the nightly melatonin rhythm is the critical parameter in photoperiodic time measurement in the golden hamster.

124 OCCLUSION OF THE MELATONIN-FREE INTERVAL BLOCKS THE GONADAL RESPONSE TO PROGRAMMED PHASIC INFUSIONS OF MELATONIN IN THE PINEALECTOMIZED MALE SYRIAN HAMSTER.

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Photoperiodic regulation of the reproductive axis is mediated by changes in the duration of the nocturnal pineal melatonin (MEL) signal. When experienced every 24 h, alterations in the duration of the signal are inevitably associated with reciprocal changes in the melatonin-free interval (MFI). Infusion of Syrian hamsters with MEL for 4 h per night, which corresponds to a MFI of up to 20 h, has no effect on gonadal function whereas a 10 h signal, equivalent to a MFI of up to 14 h leads to gonadal atrophy. A 10 h MEL signal becomes less effective when paired with progressively longer MFIs. These findings raise the hypothesis that photoperiodic time measurement may depend not only on an ability to time the MEL signal, but also to recognise the length of the intervening MFI. As a first test of this hypothesis, this study investigated the effect of occlusion of the MFI on the gonadal response to programmed MEL signals. A significant involvement of the MFI would be mainfested by a loss of responsiveness to phasic MEL signals in which the MFI was obscured by continuous infusion.

PX animals received infusions of MEL or saline vehicle via chronic s.c. cannulae attached to a programmable infusion pump. The infusion patterns were; (a) Nightly infusion lasting 10 h (25 ng MEL in 50 µl saline/h) (b) Continuous infusion over 24 h (25 ng/h) (c) Compound infusion, combining a and b with a maintained baseline of 25 ng/h stepped up for 10 h every night to 50 ng/h. (d) Large compound infusion, maintained baseline 25 ng/h stepped to 75 ng/h for 10 h. After 6 weeks of infusion, all saline groups had large testes (mean paired testes weights (±s.e.m.) between 2.79±0.20 and 3.29 ±0.35 g). Nightly MEL infusions induced gonadal atrophy (0.24±0.05 g). However, animals which received continuous or compound MEL infusions had large testes (Group b 2.95±0.35; c 2.76±0.34; d 2.62±0.25). Occlusion of the MFI blocked the gonadal response to a phasic MEL signal. These data are consistent with the hypothesis that the MFI is a necessary component of the photoperiodic stimulus.

125 PHOTOPERIODIC MODULATION OF SEASONAL OBESITY IN HAMSTERS: ARE CORTISOL AND PROLACTIN PHASE SHIFT INVOLVED? Katarina T. Borer, Pamela Johnson, Mary Beth Brosammer, Uma Swamy, and Melva V. Thompson. Dept. of Kinesiology, Univ. Michigan, Ann Arbor, MI.

We tested the hypothesis that the seasonal change in daylight produces altered phase relationships of circluating prolactin (PRL) and cortisol (C), and that this endocrine change produces seasonal changes in hamster body fat content (A.H.Maier, J.Endocrinol.1985,106:173). We expected that LD and SD photoperiods would produce differences in the phase relationships of PRL and C, and in plasma concentration of insulin. Mature female hamsters were assigned to 4 groups: SD (8L:16D, lights on at 0700, n=7), RSD (8L:16D, lights on at 1700, n=7), LD (16L:8D, lights on at 0700, n=7), and RLD (16L:8D, lights on at 2100, n=7) for a period of 57 days at which point they were implanted with chronic ic catheters, and sequentially bled 24 h later at 20 min intervals between 0830 and 1600 h. Concentrations of PRL, C, and insulin were measured, respectively by a homologous RIA for hamster PRL, with Corta-Kit, and RIA for porcine insulin. Body fat content was determined by petroleum ether extraction of carcass homogenates. Hormone data were subjected to PULSAR analysis. In addition, 2 x 2 ANOVA was performed on averages of hormone data during corresponding time periods. Estrous cyclicity was measured during the first two and last two weeks by Orsini procedure of vaginal secretion.

Photoperiod was associated with a significant difference in circadian secretory pattern of PRL, C, and insulin. In SD hamsters, PRL remained below 10 ng/ml between 0900 and 1230 h and rose to highest values between 1530 and 2045 h while C peaked between 2330 and 0930 h, a 10 h phase difference. In LD hamsters, highest PRL values were found at 1200-1330 and 2300-030 h and C peak at 1230-1400, in phase with diurnal PRL release. Plasma insulin fluctuated between 2 and 3 ng/ml at all times except at 2200-0330 h in LD hamsters when it averaged 5.04 + 0.2 ng/ml with two episodes of heightened release. All hamsters remained cyclic, and final body weight and body fat of SD and LD hamsters did not differ.

126 REPRODUCTIVE HISTORY AND THE NEUROENDOCRINE RESPONSE TO DAYLENGTH.
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Previous studies have demonstrated the importance of photoperiodic history in determining the reproductive response of the ewe to a specific daylength (J. Reprod. Fer. 80.159-165,1987). In those studies, however, the effects of photoperiodic history and reproductive state were not separated. The current study tested the hypothesis that response to a specific photoperiodic signal differs with reproductive history. Our approach was to make Suffolk ewes nonphotoperiodic by pinealectomy. As a consequence, they had no recent photoperiodic history and were expressing desynchronous reproductive cycles. The ewes were then challenged at different phases of their cycles with a long-day signal for 35 days. Specifically, ewes pinealectomized at least 1-1/2 years previously, received programmed infusions of a 24-hr pattern of melatonin which simulated that secreted on the summer-solstice in pineal-intact ewes (8 hr infusion/24 hr). Each ewe was ovariectomized and treated sc with a Silastic estradiol implant; reproductive status was assessed by serum concentrations of luteinizing hormone (LH). Changes in LH levels were identified by a cluster analysis. Clusters of high LH values reflect reproductive induction, low LH, inhibition. To test the hypothesis, that reproductive state affects photoperiodic response, melatonin was infused into 12 ewes in different reproductive conditions (6 ewes high LH, 2 ewes transition low to high, 4 ewes low LH). Controls (n=8) did not receive melatonin. During the infusion, LH remained low in the low LH group, and declined in the high LH and transition groups. Of particular interest was the reproductive response after termination of the long-day infusion. All ewes exhibited synchronous LH rises approximately 50 days later (44 ± 5 days for high LH; 49 ± 8 days for transition; 53 ± 4 days for low LH). Such a synchronous LH rise was not observed in the control group. Thus, 35 days of a summer-solstice pattern of melatonin synchronized the onset of an LH rise in long-term pinealectomized ewes, and reproductive status at the start of treatment did not affect this acute photoperiodic response. It must be stressed, however, that our study did not determine whether there were any long term effects of melatonin treatment on the endogenous reproductive rhythm. Our findings are consistent with the conclusion that the acute reproductive response to a specific photoperiodic signal, in this case an LH rise in ewes following termination of a long-day cue, does not vary with reproductive history. NSF-DCB 8710099.

127 THE DEVELOPMENT AND ENDOGENOUS NATURE OF SEASONAL RHYTHMS IN DEER.
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Adult red deer exhibit overt seasonal rhythms of metabolic rate, food intake (FI), growth rate, pelage growth, moult and reproduction. We examine how these rhythms develop over the first 18 months of life. 4 groups of female red deer were hand-reared from birth in June 1988 and weaned at 120 days of age. Group 1 (n=7) were maintained on natural photoperiods, Group 2 (n=6) were transferred from natural photoperiods to summer solstitial photoperiods at the first winter solstice, Group 3 (n=8,) were maintained on summer solstitial photoperiods from June 22 1988 and Group 4 (n=6) were treated with s.c. melatonin implants from the first winter solstice. Individual FI was measured daily, body weight weekly and twice weekly blood samples were taken for hormone determination. Serial blood samples (x 15 mins) were collected around the solstices and equinoxes for the measurement of LH and GH pulses. In Group 1, FI declined from November 1988, when determinations began, to a nadir in early February, rising to a peak by early July and declining again from early September. In Group 2, FI increased from the time of transfer to LD in late December to a peak in mid-April and declined from early June, both significantly earlier than Group 1. Group 3 exhibited a similar annual FI cycle to the controls with the phase of the cycle delayed by 4-6 weeks. In Group 4 the rise in FI was delayed by approximately 6 weeks. Changes in the pattern of coat moult, plasma prolactin concentrations and the pulsatile secretion of growth hormone were associated with the phase of the FI cycle in each group. These data demonstrate: 1. seasonal physiological and endocrine rhythms can be entrained by photoperiod and that the long-day photorefractory mechanism is present from at least 6 months of age, 2. The normal rise in FI in February is photoperiod mediated and not a consequence of photorefractoriness, 3. endogenous rhythms are expressed in animals maintained on LD from birth. These data suggest that potent seasonal rhythms seen in long-lived temperate mammals such as deer may be entrained pre-natally via a transplacental melatonin signal.

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ENDOGENOUS CIRCANNUAL RHYTHMS AND PHOTOREFRACTORINESS OF PROLACTIN SECRETION, TESTIS ACTIVITY AND MOULT IN THE MINK.

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Mink are seasonal photodependant breeders. Testis (T) recrudescence occurs when daylength is less than 12h. The spring moult beginning in April and the autumn moult in September are triggered by increasing and decreasing prolactin (PRL) secretion, respectively, secretion which is suppressed by daylength less than 12h. To determine whether these rhythms are entrained by the annual changes in daylength or whether they result from the synchronisation of an endogenous rhythm, male mink were maintained under constant conditi temperature and photoperiod, either 14°C and LD 8:16 or 18°C and LD 16:8 for 4 years. After 10 weeks of short days, T regressed and did not display any more recrudescence. Under long days, mink remained permanently sexually inactive. On the contrary, body weight, plasma PRL levels and moulting periods exhibited cycles with periods ranging between 11 and 16 months, the recurrence being clearer under short than long days.

Photorefractoriness under short days leading to spontaneous T regression was observed in the above experiment. To determine whether mink requires long days to break off this photorefractoriness, males were transferred from outside to short days on May 15, just when regression was completed, June 15 or July 3. T recrudescence was observed from early September in 1/10, 5/10 and 5/8 males, respectively. In the 3 groups, a sharp decrease in plasma PRL levels occured 3 to 7 days following the transfer under short days and the onset of the autumn moult began on August 3, 8 and 13.

These results show i) the existence of an endogenous circannual rhythm in prolactin secretion under long days but also under inhibitory short days, rhythm which may be responsible for the recurrence of moulting periods ii) the absence of such a rhythm in T activity iii) a requirement of long days to break off the refractoriness of T activity to short days, but the absence of this requirement for PRL secretion. So endogenous rhythms or photorefractoriness are not necessarily expressed for all the functions.

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OPTIMAL LIGHT SENSITIVITY - ITS SIGNIFICANCE FOR MEASUREMENT OF DAYLENGTH

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In an experiment on domestic canaries (*Serinus canaria*), it was shown that relatively small daily changes in light intensity between 10 and 12 lux were effective in entraining the free-running rhythms of perch-hopping and feeding activity(1). The data presented here verify this result and further document that in the range of about 10 lux the circadian system is maximally responsive to such small changes (highest percentage of entrained birds), while at lower and higher mean intensities similar amplitudes of light intensity changes have smaller or no effects. One possible advantage of a differential response to light intensity changes may be related to the measurement of daylength (photoperiod) under conditions where the annual changes in daylength are minimal, as for example close to the equator. In many birds studied so far, photoperiodic time-measurement is based on a circadian rhythm of photosensitivity. A special version of the "external coincidence" model would imply that, when light intensity changes within a critical range coincide with the sensitive phase of this rhythm, the photoreceptive system responsible for entrainment of the circadian pacemakers responds maximally. A bird could thus discriminate between small changes in daylength (1 h or less). Experiments are presently being conducted to test this hypothesis.

(1) Pohl, H. Die Vogelwarte 34, 291-301, 1988.

130 STIMULATION OF REPRODUCTION IN SEASONALLY REPRODUCTIVELY REGRESSED ANIMALS BY DIETARY DIHYDROXYPHENYLALANINE. John M. Wilson and Albert H. Meier, Dept. Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

The annual reproductive cycle of the Syrian hamster is divisible into two parts by the manner of response to short daylengths (<12.5 hours). Short daylengths are inhibitory to the reproductive system in scotosensitive (SS) hamsters in fall and winter, but not in scotorefractory (SR) hamsters in spring. Dihydroxyphenylalanine (DOPA) concentrations were determined by high-pressure liquid chromatography with electrochemical detection in the anterior hypothalamus and plasma of SS and SR hamsters maintained on short daylengths (LD 10:14). Anterior hypothalamus and plasma concentrations of DOPA were nearly two-fold greater in SR than SS hamsters. Accordingly, SS male hamsters that had undergone reproductive regression on short daylengths were provided DOPA in the diet. After 6 weeks of treatment, testis index and mating success of DOPA-treated males was similar to that found in naturally SR hamsters. However, control SS males were ineffective at mating and their testis indices indicated nearly complete gonadal regression. DOPA feeding also maintained estrous cycling and mating success in SS female hamsters transferred to short daylengths. The effects of DOPA feeding on reproductive responsiveness to daylengths was also tested in Japanese quail. DOPA treatment stimulated reproductive readiness in reproductively regressed (photorefractory) quail. Thus, DOPA-feeding may reset the endogenous annual mechanism in a manner similar to the natural alternation of seasonal conditions.

131 THE EFFECT OF PHARMACOLOGICAL MANIPULATION OF CALCIUM CHANNELS ON *IN VITRO* 2-DEOXYGLUCOSE UPTAKE OF THE SCN. V.H. Cao, J.D. Miller, and T. Kilduff, Depts. of Biological Sciences and Psychiatry, Stanford University, Stanford, CA 94305

Exposure to low calcium media *in vitro* nearly abolishes the circadian rhythms of neuronal firing and 2-deoxyglucose (2DG) uptake in the suprachiasmatic nuclei (SCN) (Shibata et al.,1987). These findings suggest that circadian clock function may require calcium-dependent neurotransmitter release. This study attempted to determine the nature of the calcium channel necessary for the diurnal elevation in relative 2DG uptake (R2DGU) in the SCN through the use of selective calcium channel (L, N, or T) antagonists.

SCN slices (500 μ) from male Wistar rats, entrained to LD12:12, were prepared in standard fashion (Newman & Hospod, 1986) around CT2. Experimental slices were pre-incubated in buffer (75 min) followed by buffer plus drug (30 min), whereas control slices were exposed only to the buffer (105 min). Slices were then placed in incubation buffer containing [^{14}C] 2DG, with or without drug, respectively, for 45 min. Slices were then rinsed (30 min) in the presence of drug where appropriate, frozen, sectioned, and autoradiographed. Sections through the SCN were chosen on the basis of Nissl stain and the corresponding autoradiograms were analyzed by an image analysis system. Bilateral measurements were made at the dorsomedial and ventrolateral poles of the SCN, the adjacent anterolateral hypothalamus (AH), and optic chiasm. Bilateral R2DGU values from the SCN, normalized to the AH, were averaged across sections to give subject scores.

The L channel calcium channel blocker, verapamil, was without effect on R2DGU in the SCN or optic chiasm ($p>0.19$). The N channel blocker, ω-conotoxin, actually produced a small, but significant, elevation (approximately 16%, $p<0.04$) in R2DGU in the dorsomedial SCN of the drug group ($n=13$) compared to controls($n=9$) and was ineffective elsewhere ($p>0.11$). Thus, it is probable that the effects of low calcium in the SCN slice are mediated by a novel calcium channel that is not sensitive to verapamil or ω-conotoxin. Our observations are supported by the recent finding that ω-conotoxin binding in the hypothalamus is minimal. Ongoing studies are examining T channel blockade and the effects of inorganic divalent cations on SCN metabolism (Supported in part by a grant from the Upjohn Company).

132 REGIONAL VARIATION IN 2-DEOXYGLUCOSE UPTAKE IN THE SCN: EFFECTS OF VIP ANTAGONISM. H.C. Heller, J. D. Miller, V. H. Cao, and T. S. Kilduff. Depts. of Biological Sciences and Psychiatry, Stanford University, Stanford, CA 94305.

Previous studies have demonstrated an anterior-posterior gradient in relative 2-deoxyglucose uptake (R2DGU) in the SCN (Schwartz et al., 1987). Immunohistochemical studies have shown that vasopressin neurons are relatively restricted to the dorsomedial SCN, whereas vasoactive intestinal polypeptide (VIP) neurons are relatively restricted to the ventrolateral region. In this study we have examined R2DGU in both the dorsomedial and ventrolateral regions of the SCN in vitro. Furthermore, we have studied the effects of the VIP antagonist,4-Cl-D-Phe^{6},Leu^{17} VIP, (10μM and 30μM) on R2DGU in these regions.

SCN slices (500 μ) from male Wistar rats, entrained to LD12:12, were prepared in standard fashion (Newman & Hospod, 1986) around CT2. Experimental slices were pre-incubated in buffer (75 min) followed by buffer plus drug (30 min), whereas control slices were exposed only to the buffer (105 min). Slices were then placed in incubation buffer containing [^{14}C] 2DG, with or without drug, respectively, for 45 min. Slices were then rinsed (30 min) in the presence of drug where appropriate, frozen, sectioned, and autoradiographed. Sections through the SCN were chosen on the basis of Nissl stain and the corresponding autoradiograms were analyzed by an image analysis system. Samples were taken at the dorsomedial and ventrolateral poles of the SCN bilaterally, adjoining anterolateral hypothalamus (AH), and optic chiasm. Relative 2DG uptake (R2DGU) scores from the SCN regions, normalized to the AH, were averaged across sides and sections to give subject scores.

R2DGU scores in the dorsomedial SCN were significantly higher than corresponding scores from the ventrolateral SCN (dSCN $\bar{x} \pm$ S.E. = 1.45 $\pm$ 0.05, vSCN $\bar{x} \pm$ S.E. = 1.28 $\pm$ 0.03; n = 42; $p<0.001$). Exposure to the VIP antagonist at either concentration was without effect on R2DGU in either SCN region or in the optic chiasm ($p>0.6$; n = 6 for both groups). These results suggest that diurnal release of VIP in the SCN may be unrelated to either SCN R2DGU in general or regional patterns of 2DG uptake in the SCN. (Supported in part by a grant from the Upjohn Company.)

133 QUIPAZINE (A SEROTONIN AGONIST) PHASE-SHIFTS THE MAMMALIAN CIRCADIAN CLOCK IN VITRO. R.A. Prosser, J.D. Miller, and H.C. Heller. Dept. of Biological Sciences, Stanford University, Stanford, CA, 94305.

The mammalian circadian clock located in the suprachiasmatic nuclei (SCN) survives isolation in a brain slice preparation, where it continues to produce a 24 hr rhythm in spontaneous neuronal firing. Previous work has shown that the phase of the SCN clock can be shifted by a variety of *in vitro* treatments that stimulate second messenger pathways. We are currently investigating the ability of serotonin to modulate the phase of the SCN clock.

For these experiments, 500 μm brain slices containing the SCN were prepared from male Wistar rats housed in 12:12 LD. The slices were maintained in a brain slice chamber under constant perifusion conditions, as described previously (J. Neurosci. 9: 1073). At various circadian times (CT) the slices were treated for 1 hr with perifusion medium containing the non-specific serotonin agonist quipazine (10 μM). The firing rates of single SCN neurons were then recorded extracellularly during the subsequent circadian cycle (recording from each cell for 5 min). The firing rates were then averaged over 2 hr intervals to determine the time-of-peak for the population of neurons. This time was then compared to the time-of-peak of untreated slices (CT 5.95$\pm$ 0.41, where CT 0= lights-on in the donor colony; N=4) to determine the amount of shift induced by the treatment.

Quipazine treatment during mid-subjective day (CT 6, and 9) induced phase advances ($\bar{X}$= 3.62$\pm$0.41; N=3), whereas treatment during the subjective night (CT 15 and 21) induced phase-delays ($\bar{X}$= -2.97$\pm$0.8; N=3); treatment at CT 0, 3 and 12 had little effect on the time-of-peak ($\bar{X}$= 0.08$\pm$0.91; N=3). These results suggest that the SCN clock is directly sensitive to serotonergic stimulation. Further, the pattern of SCN sensitivity is similar to that seen with a variety of *in vivo* treatments, including dark pulses and induced wheelrunning activity.

134 EFFECTS OF THE NORADRENERGIC NEUROTOXIN DSP-4 ON FREE-RUNNING CIRCADIAN ACTIVITY RHYTHMS. Alan M. Rosenwasser, Dep't of Psychology, University of Maine.

The alpha-adrenergic agonist, clonidine, alters the free-running period, the amplitude, and the level of free-running circadian activity rhythms. Although clonidine is generally considered to act at the alpha$_2$ noradrenergic autoreceptor, it is clear that this agent produces physiologically-relevant stimulation of both pre- and postsynaptic alpha$_2$ and alpha$_1$ receptors, and that some clonidine effects are at least partially mediated by non-adrenergic neurons. The present study used the highly selective noradrenergic neurotoxin to further test the involvement of the noradrenergic system in circadian activity rhythms, and to begin to isolate the mechanisms underlying the effects of clonidine on activity rhythms. Rats were maintained in running-wheel cages in continuous darkness throughout the study. Activity rhythms were monitored for about four weeks before and after treatment with either DSP-4 (50.0 mg/kg, i.p.) or saline vehicle. Animals in both groups were then studied during about four weeks of clonidine administration (5.0 ug/ml) via the drinking water, and finally, for about four weeks after the termination of clonidine treatment. DSP-4 shortened the free-running period of the activity rhythm, but had no consistent effect on either the amplitude or the level of the rhythm. In addition, DSP-4 partially blocked the period-shortening effect of subsequent clonidine administration, suggesting that the effects of clonidine on free-running period are partially or completely dependent on stimulation of presynaptic adrenergic receptors. In contrast, DSP-4 had no significant effect on the relative hypoactivity induced by subsequent clonidine treatment. Surprisingly, DSP-4 appeared to largely block the amplitude-reducing effect of clonidine, even though DSP-4 itself failed to alter this characteristic of the activity rhythm. These results imply that clonidine may act primarily at post-synaptic receptors to alter the level of activity, and may act at both pre- and post-synaptic sites to alter the amplitude of the activity rhythm.

135 CHLORDIAZEPOXIDE-INDUCED PHASE ADVANCES IN SYRIAN HAMSTERS: A BEHAVIORAL AND IN VITRO ELECTROPHYSIOLOGICAL STUDY. S.M. Biello, M.E. Harrington & R. Mason* Department of Psychology, Smith College, Northampton, Ma 01063 USA & Department of Physiology, Medical School, Queen's Medical Centre, Nottingham, UK.

Administration of benzodiazepines at the appropriate time in the circadian cycle has been shown to induce phase-shifts in the circadian locomotor activity in hamsters (Turek & Losee-Olson, 1986, Ralph & Menaker, 1989, Wee & Turek, 1989). The present study examined the effects of the benzodiazepine chlordiazepoxide (CDZ) on circadian behavior and SCN electrophysiology.

Male Syrian hamsters (n=21) were housed individually and their wheel running behavior was monitored continuously by a computer system. Following fourteen days under LD 10:14 hamsters were allowed to freerun in constant dim light for two weeks. The animals then received an injection of saline vehicle or CDZ (.05-200 mg/kg, i.p.) six hours prior to the onset of activity. Each animal was injected twice with an interval of six weeks between injections. Injections of vehicle alone had no phase-shifting effect on the activity rhythm. Injections of CDZ induced dose-dependent phase advances in the locomotor activity up to 90 min. Average shifts ranged from 6 minutes at a dose of 0.05 mg/kg to 135 minutes at a dose of 200 mg/kg. Four hamsters did not show a phase shift to any dose tested.

In electrophysiological studies on the SCN, raphe and intergeniculate (IGL) nuclei neurons in vitro, iontophoretic ejection of CDZ produced a dose-dependent suppression of spontaneous firing rate. When CDZ was iontophoresed with GABA, there was a potentiation of the GABA-induced inhibitory response. These CDZ-induced effects were blocked by the GABAa receptor antagonist bicuculline.

These studies indicate that CDZ is effective in phase-shifting the biological clock of most hamsters. CDZ may exert this effect directly on the SCN or via raphe and IGL projections to the SCN.

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Ralph, M.R. & Menaker, M., J. Neurosci., **9** (1989) 2858-2865.
Wee, B.E.F. & Turek, F.W., Pharm. Biochem. Behav., **32** (1989) 901-906.

136 CHRONIC ANTIDEPRESSANT DRUG TREATMENT ALTERS THE FLUENCE RESPONSE CURVE FOR PHASE-SHIFTING THE CIRCADIAN PACEMAKER OF SYRIAN HAMSTERS.
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Pharmacological agents used to treat depressive or anxiety disorders in humans also alter the expression of the circadian pacemaker in Syrian hamsters. We have previously demonstrated that inhibition of type A monoamine oxidase with clorgyline or deprenyl increases the circadian period of wheel-running. Similar chronic treatment with clorgyline decreases the capacity of light pulses to phase-advance the onset of wheel-running in Syrian hamsters. The purpose of this experiment is to fully describe the effect of chronic antidepressant drug treatment on the fluence response to light pulses timed to phase-advance the onset of wheel-running.

Hamsters were pretreated with clorgyline (2mg/kg/day s.c.) or saline for at least two weeks. Animals were then housed in LD 14.5:9.5 with running-wheels for one week before being housed in continuous dark (DD). On day 8 of DD each hamster received a 5' monochromatic light pulse (500 nm ±2 nm, 1/2 bandwidth=10nm) at CT18 and was then returned to DD for the next two weeks. Six light intensities covering six orders of magnitude (0.00137-137.0 $\mu W\ cm^{-2}$) were examined with 6-9 animals at each intensity. The magnitude of the resulting phase shift was measured by fitting a linear regression to steady-state activity onsets before and after the light pulse and determining the phase-difference between the regressions on the day after the light pulse. The data were fit to a sigmoid curve using the ALLGRF computer model. The maximum phase-shift (r_{max}) was estimated as 0.75 hours vs 1.64 hours, the half saturating light intensity (i_o) was estimated as 2.5 vs 1.25 x 10^{14} photons cm^{-2}, and the slope (r) was estimated as 0.50 vs 0.73 for clorgyline and saline treated hamsters respectively. The combination of parameters r_{max} and i_o statistically distinguished the two curves.

These results are consistent with our previous report that clorgyline decreases the capacity of light to phase-advance wheel-running. They further suggest that decreased responsiveness to light might contribute to sleep onset insomnia reported following clinical use of MAOI's. Experiments are currently in progress to measure the fluence response curve for phase delays.

137 CARBACHOL-INDUCED PHASE SHIFTS IN THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY MAY NOT BE DUE TO A DIRECT ACTION ON SCN NEURONS.
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Injection of the cholinergic agonist, carbachol (CARB) into the lateral ventricle (LV) of golden hamsters free-running in constant darkness (DD) induces dose- and phase-dependent shifts in the circadian rhythm of locomotor activity (CRLA) that are similar to those induced by light pulses (Earnest and Turek, *PNAS* 82:4277-4281, 1985; Anderson and Turek, *Neurosci. Abstr.* 11:1140, 1985). The objective of this study was to determine if CARB exerts its phase-shifting effects directly on the suprachiasmatic nucleus (SCN) by (1) determining if the amplitude of the CARB-induced phase shift in the CRLA is correlated with the proximity of the injection site to the SCN and (2) comparing the dose-response curves for the phase-shifting effects of CARB injected into the LV versus directly into the SCN region.

CARB (1.0, 3.2, 10.0, 32.0, 56.0, or 100 nmol) or the vehicle was administered in a volume of 200 nl into the area of the SCN 10 hours after activity onset to 28 adult male golden hamsters housed in DD. CARB injections of 32 nmol or larger produced dose-dependent phase advances that were significantly larger than vehicle injections. No significant correlation was found between the amplitude of the CARB-induced phase shift and the distance between the cannula tract and the SCN. Furthermore, the dose response curves for SCN and LV injections of CARB overlap, and the phase advances induced by SCN injections were similar in magnitude to those induced by LV injections. These results do not support the hypothesis that the phase-shifting effects of CARB are due to a direct action on SCN neurons. Rather, they indicate that CARB is acting outside of the SCN region to induce phase shifts in the CRLA.

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138 LIGHT AND HI K+ INDUCED PHASE SHIFTS ARE BLOCKED BY CYCLOHEXIMIDE B.L. Bogart, S.B.S. Khalsa, G.D. Block. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

Protein synthesis inhibitors have been used to perturb the circadian systems of numerous organisms. These inhibitors generate phase shifts, suggesting some role for protein synthesis in pacemaker "function"; however, a precise role for protein synthesis has yet to be determined.

Our laboratory is investigating the cellular events underlying pacemaker entrainment in the marine mollusk Bulla gouldiana. The eye of Bulla, which generates a circadian rhythm in the frequency of compound action potentials (CAPs), has proved to be an excellent model for cellular analysis. Previous work has demonstrated that the depolarizing action of light on the pacemaker cells mediates light-induced phase shifts. In this study we asked whether cycloheximide (CHX), a translational protein synthesis inhibitor, can block phase advances and delays produced by light and Hi K^+.

Our results indicate that light and depolarization induced phase shifts are blocked by CHX. Our experimental protocol was as follows: Experimental and control eyes both received a 4 hr pulse of CHX (CT20-24 or CT12-16). 1 hr after the start of the CHX treatment, experimental eyes received a 3 hr pulse of either light or Hi K^+. CHX appeared to block light phase advances at CT21-24 (3 min $\pm$ 48 95% C.I., N=6) and delays at CT13-16 (-4 min $\pm$ 89, N=6), respectively (light alone generates phase advances (69 min $\pm$ 43, N=9) and delays (-103 min $\pm$ 32, N=9) J. Comp. Physiol. 164:195). CHX also appears to block phase delays produced by 40 mM Hi K+, a depolarizing agent, at CT13-16 (-1min $\pm$ 64, N=7) (Hi K+ when applied at CT13-16 causes a phase delay in the CAP rhythm (-73min $\pm$ 34, N=8)). CHX applied alone at CT13-16 causes a phase delay (-49 min $\pm$ 58, N=3).

These findings, while suggesting a role for protein synthesis, differ form data we have obtained with anisomycin, where light induced phase delays were not blocked (Neurosci. Abstr. 14:A156.10). It is possible that the apparent blocking effect of the inhibitorsis due to the action of the inhibitor in moving the oscillator to a new phase where light or depolarization is less effective in generating the appropriate phase shift. We are currently investigating other inhibitors which may not generate phase shifts during the early and late subjective night. Supported by NS15264 GDB and NS09621 SBK.

139 RESERPINE WEAKENS THE COUPLING OF TWO CIRCADIAN RHYTHMS IN SCORPIONS
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The oscillations of two mutually coupled circadian oscillators will have preferred phase angle differences to each other. Both are locked in a distinct phase relation, which will be maintained even throughout transient changes in period length. A decrease in coupling strength between the pacemakers will result in a weakening of the mutual phase control and therefore show a less predictable distribution of phase angle differences between the overt rhythms.

In the scorpion Androctonus australis the circadian rhythm of both the electroretinogram (ERG) and locomotor activity was simultaneously registered in constant darkness (DD) for up to 120 days. Animals were placed separately in light tight Faraday cages and fixed on plexiglass plates. Platinum electrodes were implanted in the cornea of the median eyes to measure the ERG in response to a brief light flash (8ms, 550nm), and an infrared beam was used to register leg movements. The control of the recording and the data collection was performed by a microcomputer-based acquisition system. For this study the onsets of locomotor activity were plotted as a function of the subjective time of the animal using the circadian ERG rhythm as the internal time reference.

The data of the control group (n=12) in DD showed a main peak around subjective dusk (CT 9 - CT 13) and a smaller one in the early subjective day (CT 2). One interesting characteristic of this distribution was the occurrence of two 'forbidden zones' in the second half of the subjective night (CT 14 - CT 24) and the middle of the subjective day (CT 4 - CT 6), in which the scorpion rarely started to run. However, when locomotor activity did occur during these forbidden zones, there were changes in period and phase of both circadian rhythms and this lead to feedback effects of the locomotor activity on the circadian system (see abstract Hohmann et al.). The experimental animals (n=5) were injected with reserpine (10^{-5} M). Their circadian rhythms of ERG and locomotor activity had instabilities of the circadian period and a more scattered distribution of the locomotor activity onset in relation to the ERG rhythm. This suggests that a depletion of the aminergic neurotransmitters by reserpine is leading to a loss of the homeostasis of the circadian multioscillator system and a weakening of the internal coupling strength. Since no complete desynchronization of locomotor and ERG rhythm was observed, a partial phase control may also be realized by different means, possibly using the feedback pathway.

140 EVALUATION OF MELATONIN AS A MARKER OF ENDOGENOUS CIRCADIAN PHASE USING A RADIOIMMUNOASSAY (RIA) FOR MELATONIN IN HUMAN PLASMA

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Until recently, core body temperature was one of the few widely accepted markers of the output of the human circadian pacemaker. Recently, it was claimed that melatonin in peripheral circulation was also an accurate marker of circadian phase. Light-induced shifts of core body temperature phase (1) and dim light melatonin onset time (DLMO) (2) have been reported separately, but never compared.

Therefore, both markers were evaluated in studies designed to induce phase shifts. Core body temperature and plasma samples for melatonin analysis were collected throughout constant routines. Using antibody supplied by Arendt, we implemented a tritiated melatonin RIA (3) suitable for measurement of melatonin in human plasma, with 19.2+1.6% binding and an intraassay coefficient of variation of 9.2+3.6%. This method was validated by comparison with the GCMS method of Lewy (4).

We found that our melatonin RIA accurately detected elevations in melatonin levels when compared with analysis on the same samples using the GCMS method, although the RIA method is inherently limited in its ability to detect daytime decreases of melatonin to very low levels. Preliminary analysis of data from our first subject reveals a prominent circadian variation of circulating melatonin levels during 24-hour baseline conditions. Levels rose gradually after the onset of the nocturnal sleep/dark episode from a daytime average of 24 pmol/L to a nocturnal peak of 133 pmol/L at 3 AM.

These preliminary results are comparable with data on the 24-hour pattern of melatonin secretion previously reported (5), supporting the validity of our procedures. This method will now be used to compare the phase estimates using the DLMO and ECP techniques, both before and after light-induced phase shifts of endogenous circadian phase.

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141 **MELATONIN ACTS IN THE MEDIAL BASAL HYPOTHALAMUS TO CONTROL REPRODUCTION IN THE EWE.** Benoît MALPAUX, Agnès DAVEAU, Véronique GAYRARD, Françoise MAURICE and Jean-Claude THIERY, INRA, Physiologie de la Reproduction, 37380 Nouzilly, France.

The ewe is a seasonal breeder in which melatonin transduces photoperiodic information to the reproductive axis. This effect of melatonin requires an action on the central nervous system to regulate LHRH pulsatility. The present experiment was designed to localize the central site(s) of action of melatonin by determining where the insertion of micro-implants of that hormone induces reproductive changes. Twenty eight ewes were pretreated with 67 long days. Following such a treatment, ewes are known to respond to continuous elevated levels of melatonin by an initiation of reproduction. This happens 50 to 70 days after the onset of the melatonin treatment. On Day 0 of experiment, all animals remained in long days and were allocated to 5 groups. A control group (n=6) was not treated with melatonin: 3 animals were SHAM-operated, 3 were not. The animals of the other 4 groups received intracranial melatonin implants placed bilaterally in four regions: Preoptic area (POA, n=6), Anterior Hypothalamus (AH, n=4), Medial Basal Hypothalamus (MBH, n=8) and Lateral Hypothalamus (LaH, n=4). Implants were inserted within a guide cannula which had previously been implanted using a stereotaxic approach with radiographic control. Implants were made with stainless steel needles (inner diameter: 450 µm) and were filled by suction of molten melatonin. Their real position was determined at the end of the experiment by histological examination of the brain. All ewes were ovariectomized and treated with a subcutaneous Silastic implant of estradiol. Reproductive state was monitored by serum LH levels which provide an index of response to estradiol negative feedback. In the control group, LH levels remained basal (<0.6 ng/ml) until Day 149 ± 11 (Mean ± SEM; range 119-202). No difference was found between the SHAM- (empty implants in AH, MBH or LaH) and the non-operated controls. In contrast, in 4 out of 8 animals receiving melatonin in the MBH, LH levels rose above 1 ng/ml on Days 49, 55, 59 and 65. The other animals of that group were not different from controls. The implants of the four responding animals were particularly close to each other and placed near the ventromedial nucleus. In the other three groups, no increase in LH was observed before that of controls. We conclude that the sites of action of melatonin involved in the control of reproduction are within the Medial Basal hypothalamus or surrounding areas.

142 **PINEALECTOMY OF THE PREGNANT EWE DOES NOT ABOLISH THE CIRCADIAN RHYTHM IN FETAL AND MATERNAL PLASMA CONCENTRATIONS OF PROLACTIN**

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We have investigated whether the presence of a 24h rhythm in fetal and maternal plasma prolactin (PRL)[1] concentrations is dependent on the circadian melatonin signal generated by the pineal gland of the pregnant ewe.

Ten pregnant ewes were studied during winter and early spring (Southern Hemisphere: July/ September). At surgery (between 92 and 120d gestation) the pineal was removed from 4 ewes (Pinx group) and fetal and maternal vascular catheters were inserted in all ewes (Pinx and Intact). Blood samples (2ml fetal: 5 ml maternal) were collected every 2h for 24h (0900h start) in 8 experiments in each of the Pinx and Intact groups between 127 and 140d gestation. Ewes were fed once daily at 11.00h and were kept in a 12h L: 12h D cycle with lights on at 0700h. The completeness of pinealectomy was confirmed at autopsy.

The mean maternal plasma PRL concentration were higher in the Pinx group (137±13μg/l) than in the Intact ewes (55±6μg/l) but this difference was not significant. There was a significant variation in the maternal plasma PRL concentrations during the 24h periods in both the Pinx and Intact ewes and there was no difference between the two groups in the 24h PRL profile. Maternal PRL concentrations were highest between 1300h and 2300h and peaked at 2100h in the Pinx (216±70 μg/l) and Intact (109±40μg/l) groups. The mean fetal plasma PRL concentrations were significantly higher in the Pinx (35±2μg/l) than in the Intact (6.3±0.5μg/l) groups. There was a significant variation during the 24h period in fetal PRL in both the Pinx and the Intact groups and there was no difference in the 24h PRL profile between the two groups. Peak fetal PRL concentrations occurred at 2100h (44±4μg/l) in the Pinx group and at 2300h (10.5±4.0μg/l) in the Intact group.

The persistence of a 24h rhythm in fetal and maternal plasma PRL concentrations after maternal pinealectomy indicates that this rhythm is not dependent on the circadian information contained in the maternal melatonin signal.

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143 RESTRICTED ACCESS TO MOTHER SHIFTED CIRCADIAN RHYTHM OF SEROTONIN N-ACETYLTRANSFERASE (NAT) ACTIVITY IN RAT PUPS

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Although it has been well confirmed that mother can entrain the circadian rhythm of pups in both prenatal and postnatal periods, the entraining mechanism still remains unclear. To study the mechanism we investigated the postnatal entraining patterns of NAT activity rhythm in blinded rat pups subjected to restricted access to a mother during the nursing period. The complete reversal of NAT rhythm in the pineal gland took place on the 11th postnatal day, when the pups were allowed to access to a mother only during the dark peirod of 12 hours between day 5 and day 10. Either reduced hours in a day or reduced days of restricted access caused incompletion of the reversal of NAT rhythm. Neither restricted feeding time on mother rats nor nursing by a foster mother with the rhythm reversed to the original mother caused a complete reversal of the rhythm. Although melatonin injection shifted the NAT rhythm of pups, melatonin may not be responsible for entrainment of the pups' rhythm, because restricted access to a blinded and pinealectomized mother still shifted the rhythm of blinded pups. The determination of NAT rhythm in the pineal gland is a useful method to use in investigating the postnatal entraining mechanism of pups' rhythm.

144 **MELATONIN INFUSIONS IN WHEEL-RUNNING SIBERIAN HAMSTERS IN CONSTANT LIGHT: EFFECTS ON REPRODUCTIVE STATE AND HYPOTHALAMIC LHRH**

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Juvenile male ***Phodopus sungorus*** free-running in constant light and receiving long-duration daily melatonin infusions were used to address three questions: 1) Does melatonin administration alter hypothalamic LHRH?, 2) Does disruption of circadian rhythmicity in bright LL alter melatonin action on reproductive state?, 3) Does the reproductive response to melatonin infusions depend on the circadian phase of hormone administration? 18-day old hamsters were pinealectomized, cannulated and transferred from LD16:8 in wheel-running cages to dim LL (<1 lux) or bright LL (<400 lux). Hamsters received daily 10-hr infusions of melatonin (MEL, 100 ng/day) or saline (SAL) delivered either during the day (0800-1800hrs) or night (1800-0400hrs). At autopsy 12 days later, testicular weights were measured. Brains from MEL-treated hamsters with regressed testes (<80 mg) and from SAL-treated hamsters with mature testes (>300 mg) were fixed, sectioned and treated for immunocytochemistry using antisera to LHRH (Benoit LR1 and Kumar 100). LHRH-immunopositive neurons were counted within the preoptic area and hypothalamus. Initial analysis of the data indicates that MEL-treated hamsters had greater numbers of LHRH- immunopositive neurons than SAL-infused animals, suggesting a possible MEL-induced inhibition of LHRH release.

Under conditions of bright LL, the inhibitory effect of MEL on testicular growth was equally robust in day vs. night infusion groups (82 ± 32 vs. 80 ± 31mg) in contrast to SAL infusion groups (443 ± 26 vs. 436 ± 58 mg). In all hamsters, wheel-running activity was sparse and seemingly arrhythmic. Therefore, the disruption of rhythmicity due to bright LL did not interfere with the antigonadal effect of MEL. In dim LL, hamsters showed clear free-running activity rhythms with *tau* values close to 24 hrs. Interestingly, MEL infusions more consistently inhibited gonadal growth when given during the night (64 ± 16mg) than during the day (240 ± 70mg). Of 9 day-infused males, 5 had regressed and 4 had mature testes. When the phase of MEL infusions was examined relative to activity time (*alpha*) for these individuals, no consistent temporal pattern of sensitivity to melatonin could be discerned. Thus, it is not yet clear how phase or other temporal factors might influence the reproductive response to long duration melatonin infusions.

145 **MELATONIN INFUSIONS IN WHEEL-RUNNING SIBERIAN HAMSTERS IN CONSTANT DARKNESS: POSSIBLE ENTRAINMENT OF THE LOCOMOTOR ACTIVITY RHYTHM**

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Several studies were conducted to determine whether melatonin might entrain the circadian rhythm of locomotor activity in ***Phodopus sungorus***. A hormone infusion system was utilized which allows continuous measurement of wheel-running activity (for more than 6 weeks) in pinealectomized hamsters receiving daily subcutaneous infusions of melatonin (MEL, 100 ng/day) or saline (SAL). With this system, the hormone is delivered automatically without disturbance to the animal, in a pattern programmed to simulate the endogenous nocturnal pineal melatonin peak. Male hamsters 18-22 days of age were acclimated to wheel-running cages for 5 days, then pinealectomized, cannulated and transferred from LD16:8 (lights on 0200-1800 hr) to constant darkness (DD; dim red light < 2 lux). In the first experiment, animals received a 6-hour infusion beginning at 2000 hr on the day of surgery. Over the next 12 days, the duration of the infusion was gradually and symmetrically increased to 9 hours. Infusions then continued for an additional 4 weeks at the 9-hour duration (T24.0). In the second experiment, hamsters received a 10-hour infusion (T24.0) which began each day at 1600 hrs for a total of 5 weeks. At autopsy, cannula patency was tested, and testicular weights were measured to confirm the antigonadal effect of the long duration MEL infusions. Sample size was 8-10 males per group.

Data from the first experiment showed that SAL-infused hamsters free-ran in DD with a period length shorter than 24 hours (*tau* = $23.69\pm.09$ hr). Period lengths in most MEL infused males approached 24 hours (mean *tau* = $23.86\pm.07$ hr), but the difference between the two groups fell short of statistical significance ($.05< p <.1$). In the second experiment, the mean *tau* value for the MEL- infused hamsters was $23.90\pm.04$ hr, very close to the 24-hr period of the infusion and significantly greater ($p<.005$) than in the SAL group (*tau* = $23.55\pm.05$ hr). Once stabilized, activity onsets of many hormone-treated males preceded the onset of the daily MEL pulse by several hours. These data indicate that melatonin may act as a weak zeitgeber influencing the circadian pacemaker of this hamster species. Our results suggest a possible role for the high affinity melatonin binding sites which have been localized within the suprachiasmatic nuclei (SCN) of Siberian hamsters.

146 NEUROPEPTIDE-Y REGULATION OF MELATONIN SYNTHESIS IN RAT PINEALOCYTE CULTURES. James Olcese , Institute for Hormone & Fertility Research, Grandweg 64, 2000 Hamburg 54, F.R.G.

Neuropeptide-Y (NPY), a 36 amino acid peptide belonging to the pancreatic polypeptide family of regulatory peptides, has been shown to be widely distributed in the central and peripheral nervous system, where it has a diverse range of actions. NPY-immunopositive sympathetic nerve fibers to the mammalian pineal gland have been identified. Thus, the present study examined for the first time the mechanisms of action for NPY in a rat pinealocyte monolayer culture system. Significant stimulation (10 to 20-fold) of pineal melatonin secretion was achieved at NPY concentrations of 10 and 100 nM. However, in contrast to the effects of norepinephrine, this stimulation by NPY was not accompanied by significant changes in intracellular cAMP accumulation. A 12-hour preincubation of the pinealocytes with pertussis toxin (100 ng/ml) abolished the stimulatory influence of NPY, suggesting that this neuropeptide can act via a guanine nucleotide-binding protein to regulate pineal function via a cAMP-independent pathway. In light of the fact that NPY is often co-localized with norepinephrine in sympathetic fibers, the possibility exists that neural transduction in the pineal gland may involve multiple receptor systems.

147 MELATONIN INHIBITS cAMP PRODUCTION VIA A PERTUSSIS TOXIN-SENSITIVE MECHANISM. LL Carlson, DR Weaver, and SM Reppert, Lab of Developmental Chronobiology, Massachusetts General Hospital, Harvard Medical Sch., Boston, MA 02114

The pineal hormone melatonin regulates the dramatic changes in reproduction that occur in seasonally breeding mammals. Recent in vitro autoradiography studies indicate that the pars tuberalis (PT) of rodents and sheep contains high affinity melatonin receptors. In amphibian melanophores, melatonin receptor occupation decreases cAMP levels by a pertussis toxin-sensitive mechanism (White BH, et.al., J. Comp. Physiol. 157:153-159, 1987).

We examined melatonin's effects on the adenylyl cyclase system in median eminence/pars tuberalis (ME/PT) explants from sheep and Djungarian hamsters. Sheep explants were divided into twelve equal pieces and suspended in gassed (95% O_2-5% CO_2) Krebs Ringer bicarbonate buffer containing 5 mM glucose for 1 hr (37 C). Treatments were added and continued for 30 min before termination with 6% TCA. cAMP levels were measured by RIA. Forskolin (10 uM) treatment increased cAMP levels in sheep ME/PT explants, and in anterior pituitary explants. Melatonin (10 nM) inhibited forskolin-induced cAMP production in ME/PT explants by 84.2 $\pm$ 4.7%. However, melatonin was ineffective in inhibiting cAMP levels in anterior pituitary explants. This suggests that the effect of melatonin is mediated by high affinity melatonin receptors, which are located in the PT but not in the anterior pituitary.

Similar results were obtained with hamster explants. Melatonin inhibited forskolin-induced cAMP production in a dose-dependent manner; significant inhibition was present at concentrations $\geq 10^{-11}$ M. Melatonin was ineffective in pineal or anterior pituitary gland explants. Additionally, the inhibitory effect of melatonin (10 nM) was completely blocked by preincubating the ME/PT explants with pertussis toxin (1 ug/ml, 6 hr).

These data suggest that melatonin receptors in the PT area are coupled to the adenylyl cyclase regulatory system through a pertussis toxin-sensitive G protein.

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THE SITES OF MELATONIN'S ACTION IN THE BRAIN OF THE HOUSE SPARROW, PASSER DOMESTICUS. Vincent M. Cassone and David S. Brooks. Department of Biology, Texas A&M University, College Station, TX 77843.

The pineal gland and its hormone melatonin play an integral role in the circadian organization of the house sparrow, Passer domesticus. To determine the possible sites of melatonin's action within the house sparrow brain, two experiments were performed. First, the distribution of high affinity melatonin receptors were autoradiographically determined following in vitro binding of 75 pM 2[^{125}I]-iodomelatonin (IMEL). Second, the effects of 100 μg/kg melatonin at Zeitgeber time (ZT) 10 on in vivo cerebral uptake of 2-deoxyglucose-1-^{14}C (2DG) were determined autoradiographically.

IMEL binding is present in primary retinorecipient structures of the circadian, tectofugal and thalamofugal visual systems. These structures include the visual suprachiasmatic nucleus (vSCN), ventral lateral geniculate nucleus (GLv), lateral anterior nucleus (LA), dorsolateral complex (DL), ectomammillary nucleus (EM) and optic tectum (TeO). In addition, thalamic and mesencephalic visual relay nuclei, nucleus rotundus (Rt) and nucleus isthmi (Ipc), and the telencephalic tectofugal visual integrative center ectostriatum (E) bind IMEL. Other structures which bind IMEL include the habenular complex, the nucleus of Edinger-Westphal (EW), an oculomotor structure, and the robust nucleus of the archistriatum (RA), a song control center. Interestingly, IMEL binding is not found in the tuberal nucleus, the avian homologue of the arcuate nucleus, the median eminence, the pituitary or the pineal gland.

The effects of melatonin on 2DG uptake are similarly restricted to primary and secondary visual structures. Melatonin inhibits 2DG uptake at ZT10 in the vSCN, GLv, LA, DL, TeO and Rt, but not in other cerebral structures mentioned above. These data indicate that the role of the pineal gland, melatonin and the circadian system of the house sparrow may modulate not only the timing of behavior but also the sparrow's entire visual world.

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DAILY AND CIRCADIAN PATTERNS IN 2-[^{125}I]-IODOMELATONIN BINDING IN SPECIFIC SITES OF CHICK BRAIN. David S. Brooks and Vincent M. Cassone. Department of Biology, Texas A&M University, College Station, TX 77843.

Melatonin binding sites have been localized in chick brains using image analysis of 2-[^{125}I]-iodomelatonin (IMEL) autoradiography (Rivkees et. al. Endocrinology 125:363-368, 1989). In this study, we characterized the daily and circadian patterns of IMEL binding in chick brain. Newly hatched chicks, Gallus domesticus, were obtained from a local source and allowed to grow for two weeks in a 12:12 photoperiod (LD) under laboratory conditions. The daily and circadian pattern of binding was investigated over 48 hours in a 12:36 LD cycle. Five chicks were sacrificed every 4 hours beginning on Day 1 at 8 a.m. which was Zeitgeber time (ZT) 2 through ZT 22 and continuing Day 2 from circadian time (CT) 2 to CT 22. The chicks were anesthetized and perfused with 0.75% cold saline followed by phosphate buffer with 10% sucrose. Brains were removed and immediately frozen in isopentane at -20°C. The frozen brains were sectioned coronally at 20 μm on a cryostat and thaw-mounted to gelatin-coated slides. Brain sections were incubated with IMEL (Amersham Corp., Arlington Heights, IL) at varying concentrations from 5 to 500 pM for the binding characteristic study and at 75 pM for the daily binding study. Non-specific binding was determined in the presence of 1 μM melatonin. The slides were exposed to ß-Max autoradiography film (Amersham Corp.) for 7 days at -20°C. Autoradiographs were analyzed with the JAVA visual analysis system (Jandel Scientific, Corte Madera, CA), and the binding densities quantified to ^{125}I-microscale standards (Amersham Corp.). As previously shown, specific binding sites are located in several structures of the telencephalon, thalamus, hypothalamus, and midbrain of the chick including structures associated with the visual and auditory systems. Scatchard analysis of the binding indicates a low affinity and high affinity binding site, with a change in B-Max occurring in both sites from (subjective) day to (subjective) night. Analysis of data collected every four hours reveal a daily rhythm in binding sites with the peak of the rhythm occurring approximately ZT 10, while the lowest values occur at ZT 18. This rhythm persisted in DD with the high and low values occurring at circadian times 10 and 18 respectively. The source(s) of this endogenous rhythm and its consequences to avian circadian organization are currently under investigation.
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150 TEMPORAL PROFILE OF SUPEROXIDE DISMUTASE ACTIVITY IN THE PINEAL GLAND. J.Cipolla-Neto; D.S.P.Abdalla; R.P.Markus & A.Campa.Instituto de Ciências Biomédicas and Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, CEP 05508, Brazil.

Superoxide dismutase (SOD) (E.C.1.15.1.1) is part of the enzymatic antioxidant system. Several pineal enzymes (tyrosine and tryptophan hydroxylases and 2,3 indolamine dioxygenase) participate in oxygen-dependent reactions, and active oxygen species are generated by redox compounds such as cathecolamines, quinones and pteridines and by the action of MAO. Based on this we intended to study the 24h profile of total SOD (Cu-Zn-SOD and Mn-SOD) in the pineal gland of three-months old male albino rats.All animals were kept under an LD 12h:12h (lights on 06:00h) since weaning and were killed at 3h intervals throughout 24h periods.The first experimental group (LD) was always kept under the light-dark cycle; the second (LL) was transfered to a constant illumination condition (500 lux) 48h before being killed; and a third group (LDlux)was acutely illuminated during the night of the experiment (500 lux for 3h). The animals were deeply anaesthetised with ether and perfused through the left ventricule with ice cold saline. The pineal glands were rapidly removed and stored at -20oC until assayed. SOD activity was measured by the nitrite method.

The randomized block design analysis of variance (4 experimental blocks;24 rats per block;3 rats each 3h;n=96) showed a significant hour of the day effect ($p=0.009$) for SOD activity in LD group.Rhythmic analysis (best-fitting cosine curve) showed a significant 24h rhythm ($p<0.001$) with maximal SOD activity around 14:30h; a mean 24h value of 100.34+-1.60 U SOD(nitrite) per mg of protein; and an amplitude of oscillation of 8 to 14 % of the mean.In addition an ultradian rhythm of 9h was detected ($p<0.001$) with a higher peak around 13:00h and a smaller one around 22:00h. The LL group analysis of variance could not show any hour of the day effect ($p=0.251$); with a 24h mean value of 97.41+-2.37 U SOD(nitrite) per mg of protein not statistically different from the LD 24h mean. LDlux group SOD activity was greater than the activity of a control group kept in the dark ($p<0.05$, Mann-Whitney U test) and was not different from the SOD activity of the control group during the light period ($p>0.074$).

These results indicate that the greater pineal SOD total activity is in phase with the maximal gland concentration of serotonin and its metabolites derivated of the oxidation branch and that the maximal nocturnal concentration of melatonin is coincident with minimal SOD activity.

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151 **INITIAL CHARACTERIZATION OF A *XENOPUS LAEVIS* RETINAL MELATONIN DEACETYLASE.** Michael S. Grace and Joseph C. Besharse Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City KS 66103, and Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta GA 30322.

Melatonin is a component of the circadian system in the *Xenopus laevis* eye. Ocular melatonin is cleared by a deacetylase producing 5-methoxytryptamine (5MT), which is then deaminated to form 5-methoxyindoleacetic acid (5MIAA) and 5-methoxytryptophol (5MTPL). The enzyme appears to be common among vertebrate classes, although we have been unable to find activity in mammals. In order to further characterize this degradative pathway, we have developed a quantitative and reproducible assay for the melatonin deacetylase. A detergent-soluble fraction of *Xenopus* retinal membranes is incubated with [^{3}H-methoxy]-melatonin. The resulting mixture is separated by reverse-phase HPLC, and methoxyindoles are detected by fluorescence and flow liquid scintillation spectrometry. Activity is assessed by the generation of ^{3}H-5MT (5MT conversion to 5MIAA and 5MTPL by monoamine oxidase is absent under the assay conditions). Activity occurs in *Xenopus* retina, retinal pigment epithelium, and skin, all of which are affected by melatonin. The retinal enzyme appears to be membrane associated and exhibits optimal activity at approximately 37°C and pH 6. The enzyme exhibits a K_m of approximately 20μM. Both N-acetylserotonin and 5-methoxytryptamine inhibit melatonin deacetylation with similar efficacy, although they are each less effective than melatonin. This suggests that both the acetyl and methoxyl groups of melatonin are important for substrate recognition by the enzyme. Acetylcholine was completely ineffective at inhibiting melatonin deacetylation. This is an important finding since we suspected a relationship to acetylcholinesterase based upon inhibition of melatonin deacetylation by the cholinesterase inhibitor eserine. We are investigating whether the melatonin deacetylase directly contributes to the *Xenopus* retinal melatonin rhythm. These results provide an initial biochemical characterization and localization, and form a basis for isolation and further characterization of the melatonin deacetylase.

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152 THE PINEAL, THE RETINAE AND MELATONIN IN THE CIRCADIAN SYSTEM OF THE EUROPEAN RUINS LIZARD Augusto Foá, Daniel Janik*, Lucia Minutini Diparimento di Scienze del Comportamento Animale e dell'Uomo, Universitá di Pisa, Italy - *Max-Planck-Institut Fuer Verhaltensphysiologie, 8138 Andechs, FRG

The pineal organ and the eyes are undeniably important structures in the physiology of circadian rhythmicity in non-mammalian vertebrates. However, since the exact role these components play is rather species-specific, very few assumptions can be made about the underlying physiology in any species not specifically examined. Therefore, in adopting a new model organism (the ruins lizard, Podarcis sicula campestris) to understand the behavioral and ecological aspects of circadian rhythmcity in relation to physiological parameters, we found it necessary to ask old questions in this "new" species.

Locomotor activity of individual lizards was recorded in constant darkness (DD) and temperature. Pinealectomy (PINX), as well as bilateral removal of the retinae, produced marked changes in the period of the freerunning rhythm (both lengthening and shortening). The combination of both these surgeries in the same animal never abolished locomotor rhythmicity. Plasma melatonin (MEL) levels were measured by radioimmunoassay. In DD a circadian rhythm of MEL was detected in intact lizards with levels peaking at 197 pg/ml late in the subjective night. After PINX the MEL rhythm was affected in two ways: (1) peak levels were reduced to about 72 pg/ml; (2) the peak was advanced significantly. When measured at two different times of day, MEL was not detected in blood after pinealectomized lizards were subjected to bilateral removal of the retina.

Our data suggest that: (1) The pineal and the retina may influence oscillators located elsewhere in the nervous system; (2) Both the pineal and the retinae contribute to the rhythm of plasma MEL; (3) Rhythmic plasma melatonin is not required for locomotor rhythmicity; (4) An oscillatory system outside the pineal and retinae can drive locomotor rhythms.

153 PHOTOTRANSDUCTION IN CULTURED TROUT PINEAL M. Max, M. Menaker Biology, University of Virginia, Charlottesville Va.

The pineal gland of the brown trout, Salmo trutta, when maintained in suprafusion culture, synthesizes melatonin at a rate that is dependent on ambient lighting conditions and temperature. In darkness the rate of melatonin synthesis increases almost 4 fold between 9-18°c. At temperatures above 18°c synthesis declines. When glands are exposed to light the rate of melatonin synthesis decreases until it reaches a new steady state. Both the decrease in the rate of synthesis and the new steady state rate are dependent on the intensity of the light stimulus. With increasing temperature the percentage decline in melatonin synthesis produced by any particular light intensity increases in a linear fashion, but the absolute value to which melatonin falls is not significantly changed. These data indicate that the effect of temperature on melatonin synthesis in the dark differs from its effect on melatonin synthesis in the light. Forskolin blocks the decline in melatonin synthesis due to light exposure in a dose dependent fashion but does not have an effect on the rate of melatonin synthesis in the dark. Both the interaction of light and temperature and the effects of forskolin suggest that two enzymes may be rate limiting in this system; one in the dark and a different one in the light. In order to make this suggestion concrete and testable we propose that in the dark the rate of melatonin synthesis is limited by HIOMT activity whereas in the light NAT activity is rate limiting.

154 . EFFECTS OF CONTINUOUS DARKNESS ON ELECTRORETINOGRAPHIC CORRELATES OF PHOTORECEPTOR DISC SHEDDING IN RABBIT. Mary P. White and Peggy A. Hock. Division of Ophthalmology, Veterans Administration Medical Center, Palo Alto, CA 94304

When electroretinograms (ERGs) are recorded in the dark from photoentrained albino rabbits using a 500 nm test light, the b-wave decreases abruptly in amplitude, corresponding to a reduction in retinal sensitivity at about 30 min after the time of normal light onset. We have confirmed with histological observations that this change in retinal sensitivity is correlated temporally with circadian rod photoreceptor outer segment disc shedding in the albino rabbit retina. If circadian disc shedding itself produces the observed drop in retinal sensitivity, the timing of the event is predicted to be under the control of an intraocular oscillator, as shown with histological methods for rat and frog retina. To test this hypothesis we have studied albino rabbits housed in constant darkness and rabbits with unilateral dark adaptation, achieved by application of an opaque eyepatch. Histological examination of the retinas showed that in the dark-adapted eyes, including eyepatched eyes, the outer segments elongate in the superior region of the retina. In albino rabbits in cyclic lighting only short rod outer segments, 10-12 um in length, are found in all regions of the retina. After 3 or more days of dark adaptation, the superior retina contains rod outer segments 17-24 um long. Autoradiographic measurements on retinas of dark-adapted and photoentrained rabbits showed that about 2.4 um in outer segment length was displaced each day including in the elongated outer segments. Since rod outer segment disc production was constant, outer segment elongation must be due to altered disc shedding. ERG measurements showed that the retinal sensitivity decrease phase advances, whether dark adaptation is bilateral or unilateral. The timing of the decrease in retinal sensitivity was consistent with a freerunning period of 23 hr 50 min. The unpatched eye remained unaffected by unilateral dark adaptation. These data support the hypothesis that the timing of the retinal sensitivity change is under intraocular control.

155 ANALYSIS OF CIRCADIAN PHOTORECEPTORS IN RETINALLY DEGENERATE MICE. *R.G. Foster, D. Hudson, W.J De Grip* and M. Menaker. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901 USA; * Department of Biochemistry, Center for Eye Research, University of Nijmegen, NL-6500 HB Nijmegen The Netherlands.*

Mammals use their eyes for all forms of light detection but it is unclear whether visual photoreceptors - rods and cones of the retina - mediate circadian photoreception. We have addressed this question by studying a strain of genetically "blind" mice (homozygous for the rd allele; rd/rd) which show substantial and progressive destruction of the rods and cones of the retina after birth, so that by 80 days of age all that remains of the photoreceptors are a few cell bodies which lack outer segments. Our results can be summarized as follows: 1) As a measure of circadian photoreception we examined the effect on phase-shifting of a single 15 min pulse of light (515nm) at a range of radiance levels delivered at CT 16. Despite the profound destruction of the visual system in rd/rd mice 80 days of age, these animals could be phase shifted by light and the sensitivity of the response was not different from that of visually normal heterozygous rd/+ and homozygous +/+ animals; 2) We examined the anatomy of the rd/rd eye (80 - 100 days of age) using immunocytochemistry and localized a cone opsin (but not a rod opsin) within the plasma membrane of limited numbers of perikarya that lie between the pigment epithelium and inner nuclear layers of the retina. We have also identified proteins that bind the rhodopsin chromophore (11-cis retinal) within the disrupted pigment epithelium and Müller cells; 3) We used HPLC techniques to identify small amounts of the rhodopsin chromophore (11-cis retinaldehyde) and demonstrate photoisomerization of the chromophore (11-cis to all-trans) within the retina of rd/rd animals 90 - 100 days of age.

As the rd/rd mutation is progressively destructive, we are currently studying aged (>1 year) rd/rd animals to see if the circadian behavior and retinal biochemistry has altered.

156 PATHWAYS CARRYING CIRCADIAN INFORMATION THAT MODULATES ERG AMPLITUDE IN THE RETINA OF THE DIURNAL LIZARD, *ANOLIS CAROLINENSIS*

C. Raul Collazo, Andrew P. Shaw and Chester J. Karwoski
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Electroretinograms (ERGs) were recorded hourly for five consecutive days from free-moving lizards. Time-series of the ERG b- and d-waves were analyzed by computer to determine the amplitude of the circadian rhythms (CRs), as described by Fowlkes et al (1989). Several surgical procedures were used to study the processes that contribute to the generation of the ERG CRs:

(1) Bilateral Optic Nerve Transection (ONX) and Pinealectomy: Both procedures abolish the ERG CR. Amplitude is diminished by bilateral ONX, but not by pinealectomy.
(2) Unilateral ONX: Surprisingly, ERG CRs do not differ in either the transected or intact eye. However, ERG amplitude for the transected eye is slightly diminished.
(3) Tectal Lesion: A b-wave CR is present in less than half of the eyes. The d-wave CR is present, but depressed. Amplitudes are comparable to those of controls.
(4) Parietalectomy: This does not affect the ERG CR or its amplitude. Also, the ability to phase-shift to a new *zeitgeber* is not lost.
(5) Surgical Controls: These include sham-ONX, sham-pinealectomy, and forebrain lesions (which are not expected to interfere with ERG CRs). None of these procedures abolishes the ERG CR or affects ERG amplitude.

These findings suggest that the ERG CRs might be driven by a multioscillator system. Possible signals carrying the circadian information include plasma levels of melatonin (which could be rendered non-periodic after pinealectomy) and centrifugal neural signals in the optic nerve (which could be disrupted by ONX or tectal lesions).

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157 CIRCADIAN RHYTHM OF SCN NEURONAL ACTIVITY IN PHOTO-RESPONSIVE AND PHOTO-NONRESPONSIVE DJUNGARIAN HAMSTERS RECORDED *IN VITRO*. Russell R. Margraf and G. Robert Lynch. Department of Biology, Wesleyan University, Middletown, Connecticut 06457, USA.

The suprachiasmatic nucleus (SCN) is an intrinsic pacemaker responsible for initiating, regulating and maintaining a variety of endogenous circadian rhythms in mammals. While several studies have established and characterized a diurnal rhythm of neuronal firing rate in the rodent SCN, little research has been aimed at correlating SCN electrical activity with seasonal adjustments in behavior and physiology. In Djungarian hamsters, adjustments are induced by seasonal change in photoperiod and mediated by changes in the daily release of pineal melatonin. However, some hamsters fail to respond to chronic short day exposure due to possible differences in circadian organization (Puchalski and Lynch 1988 J Comp Physiol 162A:309-316). In the present experiments, SCN neuronal activity was measured *in vitro* in both photo-responsive and photo-nonresponsive Djungarian hamsters. Photo-responsive hamsters express an *in vitro* SCN neuronal firing rate of 5-8 Hz during subjective day and 1-3 Hz during subjective night. Electrical activity measured *in vitro* from SCN cells of photo-nonresponsive hamsters should maintain the higher frequency during early subjective night based on the delayed activity onset and nighttime melatonin peak reported for these hamsters (citation above). A relationship between neuronal firing rate and overt circadian behavior will provide insight into SCN organization and the role of biological clocks in regulating ecologically relevant behavior.

158 **RETINAL PROJECTIONS TO HYPOTHALAMIC, THALAMIC AND TELENCEPHALIC AREAS OF THE MINK.** J.Peytevin, L.Martinet, J.Servière.
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A direct retinohypothalamic projection (RHT) has been described in mammals; direct inputs were also observed into thalamic and telencephalic structures. In the mink (*Mustela vison*), a photosensitive seasonal breeder, these retinal projections are unknown.

Adult females received an unilateral intraocular injection of horseradish peroxidase conjugated to cholera-toxin (HRP-CT) and were killed 48 hrs later by vascular perfusion (1% paraformaldehyde, 1.25% glutaraldehyde) under deep anesthesia. Brains were serially cut (40µm) in coronal or horizontal plane. HRP products were visualized with tetramethylbenzidine reaction. The suprachiasmatic nuclei (SCN) approximately measured 1800 x 800 x 300µm (L x H x W). The largest retinal input was observed in both SCN, labelling being slightly denser in contralateral side of injection. Along rostrocaudal extent, fibers left optic chiasma (OC) from the 2nd quarter of SCN and stayed concentrated along the border of 3rd ventricle. Rostrally to SCN, a few labelled fibers left OC towards the diagonal band of Broca and pyriform cortex. Mainly on contralateral side, fibers could also be followed from OC to periventricular area, parvocellular portion of paraventricular nuclei (PVN) and from optic tract to lateral and ventral hypothalamic areas (Hyp A). A large bundle of fibers was also evident running through PVN towards thalamus. Small fibers were observed from the bed nucleus to the caudal part of stria terminalis just beneath the pineal gland.

So, in mink, direct retinal projections reached SCN and other Hyp A and extra Hyp A. Present anatomical data suggest a control of these regions by light/dark cycle bypassing SCN. Also worth noticing that photic informations reach areas close to the pineal gland. In this species, NPY containing fibers are located in the posterior and habenular commissures and seem to be connected with NPY fibers in the rostral part of the pineal (Moller et al., submitted to Cell Tissue Res.).

159 **THE SHEEP SCN: NEUROPEPTIDE/NEUROTRANSMITTER DISTRIBUTION AND RETINOHYPOTHALAMIC INPUT.** Q. Gong, R. B. Norgren, Jr., S. M. Moenter, F. J. Karsch and M. N. Lehman. Dept. Anat. & Cell Biol., U. Cincinnati; Rep. Sci. Prog., Univ. Michigan

The seasonally breeding Suffolk ewe has been a valuable model for studying reproductive responses to photoperiod. The suprachiasmatic nucleus (SCN) is thought to be important in controlling photoperiodism in the sheep and other seasonal breeders. We thus have used tract-tracing and immunocytochemical (ICC) techniques to localize and characterize the sheep SCN. ICC analyses of hypothalami of 5 adult Suffolk ewes perfused during seasonal anestrus. An immunoperoxidase procedure was used to visualize vasoactive intestinal peptide (VIP), neurophysin (NP), neuropeptide Y (NPY) and glutamic acid decarboxylase (GAD). To identify retinorecipient areas, 500 µl of 0.1% cholera toxin-HRP was injected intravitreally in 1 ewe. After a 2 day survival period, the animal was perfused and sections reacted with TMB.

VIP cells and fibers delineated an area above the optic chiasm. The rostral to caudal extent could be followed from a level where the chiasm is detached from the brain, to a dorsolateral region adjacent to the paraventricular nucleus (PVN). NPY fibers were not observed within this area although they were present in surrounding regions of the hypothalamus. Scattered parvocellular NP cells and fibers were limited to the rostral portions of the area delineated by VIP cells and fibers. GAD-positive fibers formed a heavy plexus that overlapped completely with VIP cells and fibers. Double label ICC for VIP and GAD revealed that some of the GAD fibers appear to terminate on VIP-containing cells, but most were adjacent to non-VIP cells. The entire area delineated by VIP and GAD staining contained a dense retinal projection. Sparse retinal fibers extended outside this area in a region lateral to the PVN. In summary, we have defined the sheep SCN as an area that is both retinorecipient and contains many of the same peptides/transmitters as the rodent SCN. The SCN in the sheep is situated closer to the PVN than in rodents, a fact that may have functional implications. [NIH NS28175 & HD21968 to M.N.L.]

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EFFERENT PROJECTIONS OF THE SUPRACHIASMATIC NUCLEI TO THE FOREBRAIN IN THE SYRIAN HAMSTER (*MESOCRICETUS AURATUS*). Youngstrom, T.G., Weiss, M.L. & Nunez, A.A. Michigan State University, Neuroscience Program & Dept. of Psychology, E. Lansing, Mi. 48824.

The suprachiasmatic nuclei (SCN) contain an endogenous circadian oscillator and generate a number of physiological and behavioral rhythms in mammals, including the Syrian hamster. The efferent projections of the SCN have been examined extensively in the rat revealing a number of projections arising from these nuclei and terminating in the hypothalamus and forebrain sites. In the present study, adult male and female hamsters received bilateral pressure injections of Rhodamine labeled microspheres (RLM; 60-200 nl) into several sites and were permitted to survive 7-15 days. After sacrificing the animals, the brains were sectioned in a coronal plane (60 μm), mounted to slides and examined with epifluorescence illumination. Labeled SCN neurons were evident following injections that included the anterior hypothalamic area, bed nucleus of the stria terminalis, diagonal band of Broca, preoptic area, hypothalamic paraventricular nucleus and/or thalamus (including the paraventricular nucleus, parataenial nucleus and zona incerta). The number of labeled SCN cells ranged from 2 to in excess of 20/section. Generally the cells were scattered throughout the SCN. Many of these injections also labeled retinal ganglion cells (Youngstrom & Nunez, 1989). Thus, the SCN of the Syrian hamster have efferent connections similar to those reported for the SCN of the rat. In addition, the results indicate that SCN and retinal inputs converge in several areas of the hamster brain. (Supported by NSF Grant BNS 8906576 to A.A.N. and Biomedical Research Funds from M.S.U.)

161 DAILY VARIATION IN MUSCARINIC BINDING SITES IN THE CORTEX AND HYPOTHALAMUS OF GOLDEN HAMSTERS. K. G. Bina[1], M. Wilkinson[2] and B. Rusak[1]. Department of [1]Psychology and Department of [2]Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.

Circadian variation in the binding of [^{3}H]N-methyl scopolamine ([^{3}H]NMS), a hydrophilic muscarinic receptor antagonist was studied in the hypothalamus and cortex (visual cortex) of the Golden hamster (*Mesocricetus auratus*) using a novel micropunch technique which selectively detects only cell surface (functional) receptors.

Binding sites were first characterized using 1mm punches (from slices 300 μm thick) incubated at 30^{o}C for two hours. Atropine sulphate served as the displacer. Competition curves performed in the cortex suggests that both M1and M2 receptors exist there. Intact animals maintained in LD 14:10 were killed every 4 hours starting at lights on and binding assays performed on hypothalamus and the cortex. Binding assays were also performed on SCN ablated (SCNX) animals maintained in LD 14:10 at lights on and 12 hours after lights on based on the results from the intact animals.

The intact LD animals showed a significant daily rhythm in [^{3}H]NMS binding in the cortex with highs occurring 12 hours after lights on (B Max of 4905+/-620 fmol/mg wet weight) and lows at lights on (B Max of 2270+/-394 fmol/mg wet weight). No significant rhythm was detected in the hypothalamus (1212+/-222 and 1037+/-135 fmol/mg wet weight for 12 hours after lights on and at lights on respectively). The rhythm in the cortex was abolished in the SCN-ablated animals (3317+/-528 and 4004+/-964 fmol/mg wet weight for corresponding time points). The data suggest that photic information mediated through the SCN or the pacemaker property of the SCN is responsible for this rhythm. Autoradiography ([^{3}H]NMS; 2nM, 3 weeks exposure) clearly revealed specific binding in the SCN and the surrounding periventricular region.

162 $GABA_A$ AND BENZODIAZEPAM BINDING IN ADULT AND DEVELOPING SUPRACHIASMATIC NUCLEUS. Miqun Li and Jannon L. Fuchs University of North Texas, Department of Biological Sciences, Denton TX 76203

Certain benzodiazepine and $GABA_A$ agonists or antagonists can affect rodent circadian rhythms in a phase-dependent manner, by producing phase shifts or by blocking light-induced phase shifts. To investigate whether circadian rhythms in SCN receptors might underlie the phase-dependency, we examined benzodiazepam and $GABA_A$ receptors in the rat SCN, using quantitative autoradiography of [^{3}H]flunitrazepam and [^{3}H]muscimol binding. No significant circadian changes in [^{3}H]flunitrazepam binding were found across 8 time points in LD 12:12. In addition, no differences were found between animals in light versus darkness at time points in early or late subjective night. [^{3}H]Muscimol autoradiographs from the same animals are currently being analyzed. In a developmental study, [^{3}H]flunitrazepam binding in the SCN was low on embryonic day 18 but rose to adult levels by embryonic day 20. A similar developmental change was observed for [^{3}H]muscimol. The occurrence of marked increases at the stage when fetal SCN metabolic rhythms first entrain to the maternal rhythm suggests the possibility that $GABA_A$ and associated benzodiazepine binding sites might participate in fetal SCN entrainment.

163 SEROTONIN RECEPTOR SUBTYPES IN THE SUPRACHIASMATIC NUCLEUS R.R. Dean, J.D. Miller, and W.C. Dement Stanford Sleep Disorders Center 701 Welch Rd Suite 2226 Palo Alto, CA. 94304

The mammalian suprachiasmatic nuclei (SCN), the site of the primary circadian clock, receive an exceptionally dense serotonergic innervation (Parent, Descarries & Beaudet, 1981). Recent studies in our laboratory have shown that quipazine, an antagonist at the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_3 receptors and a partial agonist at the 5-HT1C receptor (Schoffter and Hoyer, 1989), is capable of inducing phase-shifts of 3-4 hours in cultured SCN slices. Both phase advances and delays were observed depending on the circadian time of drug administration. The present autoradiographic study was undertaken to elucidate the specific 5-HT receptors involved in this effect. ^{125}I-ICYP in the presence of 30 µM isoproterenol, to mask the beta adrenergic receptors, was used to selectively label 5-HT_{1B} receptors using 1µM 5-HT or CGS 12066B to define the nonspecific binding. ^{125}I-LSD was used to label 5-HT_{1C} and 5-HT_2 receptors. 100 nM spiperone was used to mask the 5-HT_2 receptors, leaving only the 5-HT_{1C} receptors labeled and mianserin,100 nM, was used to define the nonspecific binding. The resulting data demonstrate a moderately high density of 5-HT_{1B} receptors and a complete absence of 5-HT_{1C} and 5-HT_2 receptors in the SCN of Spague-Dawley rats. Displacement studies are in progress to further characterize the 5-HT_{1B} binding in the SCN with a variety of agents with different selectivities. The 5-HT_{1A}, 5-HT_{1D} and 5-HT_3 receptors will also be investigated. This study represents the first detailed study of serotonin receptor subtypes in the SCN.

164 REGIONAL DISTRIBUTION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVITY IN THE DJUNGARIAN HAMSTER BRAIN. Marilyn J. Duncan and Willis K. Paull, Dept. of Anatomy & Neurobiology, University of Missouri Medical School, Columbia, MO 65212.

Previous studies of the Syrian hamster have shown that immunoreactivity (IR) for the astrocyte-specific protein, glial fibrillary acid protein (GFAP), is dense in the suprachiasmatic nuclei (SCN) of the hypothalamus and the paraventricular nuclei and reuniens nuclei of the thalamus (Morin, L.P. et al, Neurosci. Lett. 99:55, 1989). In conjunction with other reports that these nuclei contain 2-[125I]-iodo-melatonin binding sites (Duncan, M.J. et al, Endocrinol. 125:1011, 1989), these findings suggested that melatonin might act on glial cells. The present studies were aimed at localizing GFAP IR in the Djungarian hamster brain and investigating whether its distribution or density might be altered by changes in photoperiod which alter the duration of melatonin secretion. In Experiment 1, adult male hamsters housed in long photoperiod (16L:8D) were anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and post-fixed overnight. Serial coronal sections (30 um) through the diencephalon were cut on a vibratome and processed for immunocytochemistry using GFAP antisera (Incstar, 1:2000 dilution) and the peroxidase-antiperoxidase procedure (Sternberger, L.A. et al, J. Histochem. Cytochem. 81:315, 1970). GFAP IR was prominent in the SCN, the median eminence, the medial habenular nuclei, and the fimbria of the fornix, and also present in the stria medullaris and Ammon's horn. Control sections incubated with normal goat serum instead of primary antiserum did not show GFAP IR in any area. In Experiment 2, 18 day old male hamsters were either kept in 16L:8D or transferred to short photoperiod (12L:12D) for 14 days, and then perfused with fixative. The brains were processed for ICC; the pattern of GFAP IR was the same as seen in Experiment 1 and similar in both groups. In conclusion, GFAP IR in the Djungarian hamster is present in the SCN and several other diencephalic regions; and is not obviously altered by changes in photoperiod.

165 LESIONS OF THE SUPRACHIASMATIC NUCLEUS (SCN) THAT DISRUPT MELATONIN SECRETION MAY NOT BLOCK RESPONSES TO PHOTOPERIOD SHIFTS. G. L. Jackson, C. Kao, H. T. Jansen, Department of Veterinary Biosciences, University of Illinois

Ewes were subjected to lesions (N = 11) or sham lesions (N = 5) of the SCN in late June. They then were kept under photoperiods of 9L:15D, alternating with 16L:8D for 90- to 120-day intervals. Blood samples taken twice weekly were analyzed for prolactin and for progesterone to monitor estrous cycles. Samples taken at 30-min or 1-h intervals for 24 h on two occasions while the animals were on 16L:8D were analyzed for melatonin (M). M concentrations rose significantly and robustly in all sham ewes with the onset of darkness. Effect of lesions on M was evaluated by 2 criteria: concentrations of M during darkness in individual lesioned vs. the mean for sham ewes and difference between daytime and nighttime M in individual lesioned ewes. In 5 lesioned ewes, nighttime M was significantly less ($P < 0.05$) than mean M of sham ewes in both bleeds. In 6 lesioned ewes, mean concentrations during the night did not differ significantly ($P < 0.05$) from concentrations during the day in either 24 h bleed. In 3 of the 6, both criteria were met. In 3 of the 6 ewes, onset and cessation of estrous cycles in response to later photoperiod shifts were disrupted, and in 1 of these 3 prolactin concentrations did not change in response to photoperiod shifts. Onset of estrous cycles during the first phase of the study was not affected by SCN lesions in any ewe. These results suggest that: (1) SCN lesions that disrupt M secretory patterns in the ewe still may not affect normal responses in prolactin secretion and reproductive activity after photoperiod shifts. (2) The normally robust nocturnal elevation of M is highly redundant for effecting changes in prolactin and gonadotropin secretion. (3) SCN lesions placed in June do not appear to disrupt onset of the next breeding season but may affect subsequent responses to photoperiod shifts. (Supported by NIH HD13037)

166 PERSISTENCE OF ACTIVITY PATTERNS FOLLOWING SCN-LESIONS. P.J. Sollars and G.E. Pickard, Dept. of Anatomy, West Virginia Univ., Morgantown WV

The well-documented restoration of function observed after neural transplantation may result from any of several factors concomitant with the procedure, from a direct effect of the implant itself to a general trophic effect of the transplantation surgery. The ability of the hamster circadian system to recover its expression of rhythmicity **independently** after SCN-lesioning, was evaluated by allowing an extended post-lesion survival period.

Radio-frequency lesions aimed at the SCN were performed in 15 hamsters and wheel-running activity was monitored for approximately 200 days post-lesion while animals were maintained in constant light (red light < 1 lux in intensity). The circadian rhythm of activity was disrupted in 14 animals. Within two weeks of the lesion, the activity patterns of 13 of those animals no longer changed significantly over time, i.e., the pattern of activity, whether circadian, ultradian or arhythmic, was consistently maintained for the duration of the experiment. However, a single animal's wheel-running activity, which was arhythmic for 8 weeks after surgery, became clearly rhythmic with a circadian period similar to unoperated hamsters. The recovered rhythm persisted until the animal was prepared for histological analysis.

The extent of the remnant SCN was evaluated in all animals through the use of horseradish peroxidase labelling of retinohypothalamic processes. While none of the animals which demonstrated rhythmic behavior patterns had complete SCN lesions, several of the animals with arhythmic behavior were found to have small areas of remnant SCN tissue which were detectable only with the sensitive dark-field visualization of the HRP label.

167 CAN TIME OF IMPLANTATION AFFECT OUTCOME OF SUPRACHIASMATIC NUCLEUS TRANSPLANT? M.A. Vogelbaum and M. Menaker, Division of Biomedical Engineering and Department of Biology, University of Virginia, Charlottesville, Virginia 22901.

The mammalian SCN have been shown to contain the apparatus responsible for circadian rhythm generation. Transplantation of fetal SCN into SCN-lesioned adult hosts has restored circadian rhythmicity with periods dependent on the genotype of the donor tissue. However, the mechanism by which the implanted SCN couples to the host circadian system is unknown. Further, both the role of the SCN in what may be a hierarchy of oscillators and its mechanism of output are in question.

To examine the relationship between the SCN and its output system, we implanted τ_{ss} (period about 20 hours) fetal hypothalamic blocks into partially lesioned normal hosts with residual 24 hour rhythmicity. These implants were done either 30 days after SCN lesioning, or immediately thereafter, to evaluate the effects of recovery from neuronal injury on coupling between graft and host. When transplantation is delayed both 20 and 24 hour rhythms are expressed in the host: about 30 days after implantation, a rhythm with period of about 20 hours appears and lasts for about 10 cycles, at which point a 24 hour rhythm takes over. This alternation of rhythms continues with each rhythm lasting about 5 to 7 cycles, at which point there is a transition to the other rhythm. There is very little temporal overlap of the two rhythms, and there does not appear to be phase shifting of one rhythm by the other. When transplantation immediately follows lesion, a single rhythm is expressed with an intermediate period; a τ_{ss} hypothalamic block implanted into a partially lesioned 24 hour host produced a rhythm with a period close to 22 hours starting about 7 to 9 days after implantation. The fact that the period of this rhythm is outside the distribution of either normal or τ_{ss} periods suggests that it is the result of integration of the residual 24 hour oscillators of the host and the 20 hour oscillators in the implanted tissue.

These results suggest that a physical barrier is formed after the lesion which influences the degree of coupling between the SCN implant and the host SCN. Such a barrier could be the result of glial scar tissue which is known to prevent neural outgrowth of implanted fetal neural tissue. Perhaps implantation immediately following lesion permits neural integration whereas such integration is prevented when implantation is delayed past the point of glial scar formation.

168 **WHICH NEUROPEPTIDES/NEUROTRANSMITTERS ARE ASSOCIATED WITH SCN GRAFTS THAT RESTORE RHYTHMICITY TO HAMSTERS?** Stephen McKeehan, Eric L. Bittman, and Michael N. Lehman. Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH and Dept. Zool., Univ. Mass., Amherst, MA.

Whole tissue grafts of the fetal suprachiasmatic nucleus (SCN) which restore locomotor rhythms to SCN-lesioned hamsters contain clusters of vasoactive intestinal polypeptide (VIP) and parvocellular neurophysin (NP) cells, and associated neuropeptide Y (NPY) fibers, similar to the neurochemical organization of the unlesioned SCN (Lehman et al., *J. Neurosci.*, 7: 1626). Other transmitter systems associated with the unlesioned hamster SCN include: cholecystokinin (CCK)-positive cells and fibers (Miceli et. al., *Brain Res.*, 420: 318), and serotonergic (5HT) afferents (Card & Moore, *Neurosci.*, 13: 451).

We examined fetal SCN grafts implanted in the third ventricle which restored locomotor rhythms under constant darkness (n = 15) for the presence of CCK, 5HT, NPY, VIP, and NP in serial sections using polyclonal antisera (CCK, VIP, NP: Incstar; 5HT: Eugene Tech; NPY: gift of Dr. M. Brown) and an avidin-biotin-immunoperoxidase procedure. All grafts contained a cluster of CCK cells (10-15 μm diameter) and fibers which overlapped the location of VIP and NP clusters. Almost all CCK fibers emanating from these cells were limited to the graft with very few fibers crossing the graft-host border. In most but not all grafts, 5HT fibers of host origin entered the graft and ramified over the location of grafted VIP cell clusters. The presence of NPY input to functional grafts was also variable. In some cases, NPY fibers formed a plexus near donor VIP cells; in other instances NPY fibers which heavily innervated other portions of the graft avoided the location of VIP cell clusters. The results suggest that CCK cells and fibers comprise a component of functional SCN grafts in the hamster, but that neither NPY nor 5HT input is required for the restoration of rhythmicity after grafting. [Supported by NIH R01 NS28175 to M.N.L. and NSF BNS 8616935 to E.L.B.]

169 **RETINOHYPOTHALAMIC TRACT (RHT) AND SYNAPTOGENESIS IN THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN)**
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Synaptogenesis in the rat SCN is a largely postnatal event with the largest production of synapses occurring in the period of postnatal days 4-8 (P4-P8, Moore and Bernstein, 1989). This corresponds to the period of maximal development of the RHT (Speh and Moore, 1988) but is significantly later than the onset of functional development, as demonstrated by the 2-deoxyglucose method (Reppert and Schwartz, 1984).

In the present study, synaptogenesis and RHT development in the hamster were studied using synapsin I immunohistochemistry and anterograde transport of cholera toxin-HRP, respectively. In contrast to the rat, the hamster SCN contains numerous synapsin immunoreactive structures at P1 and is essentially comparable to the adult appearance by P4. However, there are only very sparse, scattered RHT axons in the SCN at P4, and no projections to the preoptic area (POA), anterior hypothalamic area (AHA), lateral hypothalamus (LH), retrochiasmatic area (RCA) and basal forebrain are present. By P8 projections to the SCN are more dense and projections to the POA, AHA, LHA and RCA are present. On P13 all projections are present and appear comparable to the adult (Johnson et al, 1988).

These observations indicate that there are striking differences between the rat and hamster in the timing of development of synapses in the SCN and the development of RHT projections. (Supported by NIH grant NS-16304).

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FUNCTIONAL AND TEMPORAL NEUROANATOMICAL MAPPING OF INSECT CLOCK

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We showed that the histochemical cytochrome oxidase (C.O.) staining method, which reveals changes in neuronal functional activity particularly in neuropils, was fitted to visualize rhythmic metabolic changes in the circadian pacemaker of the cockroach *Leucophaea maderae* .

This result prompted us to use this method to search a functional neuroanatomical base which could visualize some of the formal properties of photoperiodic systems, especially those related to photoperiodic time measurement. In the lepidopteran *Pieris brassicae* as in most insect species, the only established fact is that the photoreceptor as well as the clock-counter system are located in the larval brain.

Results of C.O. staining experiments showed that a precise brain structure, the larval optic centers, exhibited a change in neuronal activity following a light pulse delivered at the discrete phase points of photoperiodic sensitivity, the so-called A and B points (night-break experiment). Responses were significantly different depending on whether A, B or a neutral point without photosensitivity were considered. Comparison with controls at the same phase points, but in the dark of an unpulsed cycle showed marked differences too. Thus, in *Pieris brassicae*, the larval optic centers are a locus where the level of metabolic activity is in connection with the photoperiodic process. It must be pointed out that these structures are the anlagen of the adult optic lobes and that in some species, e.g.*Leucophaea maderae*, the circadian pacemaker was found in the optic lobes.

These results led to the assumption that, in *Pieris brassicae*, the larval optic centers could be part of the photoperiodic workshop, either as a photoreceptor or as a (part of the) clock. Results can be accomodated as well by external and internal coïncidence models.

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A REEXAMINATION OF THE ROLE OF THE NUCLEUS IN GENERATING THE CIRCADIAN RHYTHM IN ACETABULARIA. John C. Woolum, Dept of Physics and Astronomy, California State Univ. Los Angeles

Sweeney and Haxo(Science 134, 1361) reported in 1961 that the part of the Acetabularia plant containing the nucleus (the rhizoid end) could be cut off from the plant and the circadian rhythm (CR) of photosynthesis would be maintained implying that the nucleus of the plant was not involved in generating the CR. However Schweiger et al reported in 1964(Science 146, 1361) on two experiments that imply that the nucleus can infer phase information to the plant and can be therefore part of the system that generates the CR. We have repeated the second type of these experiments on Acetabularia except that the rhythm that we are studying is that of chloroplast movement up and down the stalk. Plants were placed in an entrainment chamber with an opaque plastic barrier with rhizoid end on one side and the rest of the plant on the opposite. The two sides of the chamber were exposed to opposite light dark cycles (12:12). The plants were transferred to constant light a few minutes before the change in the cycle. Transmission of light through the base of the plant was measured every minute and averaged every hour. The hourly averages were recorded and plotted so phases could be determined easily to within a few hours. The results with about 20 plants show considerable variations. Often the first day or so the rhythm was not circadian but after that the rhythm would settle down to a typical circadian rhythm with a period similar to that of the controls (about 26 hours). In most cases the ultimate phases were closer to the phase on which the apex end was entrained than to that on which the rhizoid end was entrained(opposite of the results of Schweiger). Similar experiments were done with plants with no rhizoids i.e. about half the rhizoidless plant was on each side of the opaque entrainment barrier. Phases obtained with this type of experiment were nearly random. Our results seem to imply that the nucleus is not able to impart phase information to the circadian oscillator. The phase may be determined by the fractions of the total plant on the two sides of the barrier.

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REGULATION OF GENES UNDER CONTROL OF THE CIRCADIAN CLOCK

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It is clear from a cursory examination of the biology of circadian rhythms that a wide variety of processes are being controlled, directly or indirectly, by these clocks. Doubtlessly one important aspect of this temporal control within a rhythmic cell's metabolic network is the daily control of gene expression. We are using the *Neurospora* clock system to study the flow of information from the clock to timed target genes- "clock-controlled-genes"- whose levels of expression are controlled by the clock. As a result of these studies we will also be able to develop a selection scheme for the isolation of novel clock-mutant strains and suppressors of existing strains. As a first step, we undertook the systematic isolation of morning and evening specific genes through the use of subtractive and differential hybridizations. Unexpectedly, we have reproducibly identified only three genes that are strongly regulated by the clock at the level of mRNA abundance (Loros *et al*, Science,*243*, 1989 and unpublished).

By Northern analysis over two circadian cycles, the abundance of the mRNA's have been shown to oscillate with a period of 22 hours in a clock wild-type strain and 29 hours in the long period clock mutant *frq*^{7}. Two of these genes, *ccg-1* and *ccg-2*, are specific to the subjective morning while the other, *ccg-3*, is evening specific. We have developed a method for transcriptional analysis by nuclear run-on assay in *Neurospora* and used it to show that regulation of mRNA abundance for the two morning specific genes is chiefly or wholly at the level of transcription. Sequence analysis of genomic and cDNA clones for the *ccg-1* gene has been completed allowing a complete description of the transcription unit. Within these regions we are identifying the cis-acting regulatory sequences responsible for clock control of mRNA abundance. Data describing the localization of these sequences and their use in the development of the mutant selection/enrichment scheme will be presented.

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PORTIONS OF THE NEUROSPORA frq PROTEIN SHOW SIMILARITY WITH NUCLEAR LOCALIZATION SIGNALS.

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The frq locus of Neurospora crassa has been cloned and partially sequenced, and an open reading frame that codes for 788 C-terminal residues of the frq protein was identified [McClung, C.R., Fox, B.A., and Dunlap, J.C., Nature 339, 558-562, 1989]. Previous computer-assisted analysis of the sequence showed no homology with any known amino acid sequence but did show weak similarity with the per clock protein of Drosophila melanogaster. Several possible functional features of this sequence were noted - a protein kinase C phosphorylation site, three cyclic AMP-dependent protein kinase A phosphorylation sites, several O- and N-linked glycosylation sites, and a PEST region [J. Dunlap, personal communication]. PEST regions have been associated with proteins that show high turnover rates.

We undertook a search for additional clues to the function of the frq protein. Our analyses reveal three additional features: 1) two strings of basic amino acids (residues 361-367 and 437-443) similar to nuclear localization signals described in the SV40 large-T antigen and nucleoplasmin, 2) several sequences that fit the description of casein kinase II phosphorylation sites in having either serine or threonine followed by clusters of acidic residues, and 3) acidic regions that could act as transcriptional activating regions as in the yeast regulatory proteins GAL4 and GCN4, as activity modulating domains as in the yeast RAD6 protein, or as sites for protein-protein interactions as proposed in both nucleoplasmin and nucleolin. A search for possible DNA-binding domains showed no regions of sequence similarity with any known DNA-binding motifs. With other genetic and molecular data we speculate that frq could be a nuclear regulatory protein whose activity is also highly regulated.

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174 MOLECULAR ANALYSIS OF THE *FREQUENCY* AND *PERIOD-4* LOCI OF *NEUROSPORA*.
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The *frq* (*frequency*) locus is thought to be of central importance to the biological circadian clock of *Neurospora crassa* and therefore represented an important initial target for the molecular dissection of the *Neurospora* clock. *Frq* has been localized on linkage group VII R (Loros, Richman, and Feldman, 1986 Genetics 114:1095), allowing its cloning via a 200 kbp chromosome walk (McClung, Fox, and Dunlap, 1989 Nature 339:558). The *frq* locus gives rise to two transcripts 1.5 knt and 5 knt in length and is contained within a 7.7 kbp region of DNA, the sequence of which has been determined. Within the large transcript there is a long open reading frame (LORF) potentially encoding 788 amino acids. Part of this LORF encodes a region of threonine/glycine and serine/glycine repeats that shows similarity, both at the nucleotide and amino acid levels, to *Drosophila per* protein. Other similarities among *frq*, *per*, and other proteins include but are not limited to a series of potential N- and O-linked glycosylation sites, potential kinase A and C phosphorylation sites and a PEST sequence which may signal rapid turnover of these proteins (Dunlap, Trends in Genetics, in press). cDNA sequences from the region of the *frq* transcripts have now been determined and allow a more complete analysis of the size, domain characteristics, and composition of the *frq* protein(s).

A similar approach has been used to clone DNA from linkage group I R corresponding to the *period-4* (*prd-4*) locus, the single mutant allele of which is characterized by a 3.5 hour shortening of the circadian clock. *Prd-4* was mapped between *arg-13* and *os-1* on linkage group I R. *Arg-13* was cloned by sib selection (Vollmer and Yanofsky, 1986 PNAS 83:4869) to allow an entry point for a directional chromosome walk of approximately 100 kbp across this region. DNA corresponding to *arg-13*, *os-1*, and *prd-4* has been isolated and subcloning is in progress. In heterokaryon analysis, *prd-4* has been shown to be nearly dominant, in contrast to results typically seen with other clock genes.

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CIRCADIAN RHYTHMS IN *NEUROSPORA*: PHASE-RESPONSE CURVES AND OSCILLATOR AMPLITUDE IN CLOCK MUTANTS

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Phase response curves (PRCs) were obtained for the effects of light and temperature pulses on the spore-forming (conidiation) rhythm of two different long period strains of *Neurospora*. The *cel* strain (fatty acid synthetase deficient) has a 40 hr period when supplemented with the fatty acid, linoleic acid. Under these conditions, Type I (weak) PRCs were obtained for both the light pulses (200 foot-candles for 1 hr) and temperature pulses (22°C → 40°C for 1 hr). The same strain, when supplemented with palmitic acid had a 20 hr period, and gave Type 0 (strong) PRCs for both stimuli. The control strain (*cel*$^+$) with a 21 hr period also gave Type 0 PRCs for both stimuli.

The *cla*-1 strain, which is associated with a reciprocal translocation between linkage groups I and VII, had a period of 28 hrs and Type 0 PRCs for both stimuli. However, the maximum phase changes for both stimuli were less than for the control *cla*$^+$ (21 hr) strain tested under the same conditions.

Since the *cla*-1 strain, with a 28 hr period, showed decreased responses to both phase-resetting stimuli, while the *cel* strain, with a 40 hr period showed even greater dimunition in its responses, there appears to be a correlation between period and phase resetting. Since decreased responses were found for two very different stimuli, they are unlikely to result from effects of the mutations on just photoreceptors or thermal sensors. Rather, we offer as a model, the proposal that the *Neurospora* oscillator is a limit cycle and that the mutations increase the amplitude of the oscillator, leading to a dimunition of the phase-shifting response to these stimuli and an increase in the period. This interpretation can also explain the finding of others that certain long period mutants of *Neurospora* such as *frq*-7 (29 hrs) show decreased responses to phase-resetting stimuli, such as light, temperature and cycloheximide. The model predicts that short period mutants will show increased responses to these same stimuli.

176 IMMUNOHISTOLOGICAL STAINING OF A *PER*-LIKE PROTEIN IN THE BEETLE, *PACHYMORPHA SEXGUTTATA*

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The *per* gene of *Drosophila* influences locomotor rhythms of adults as well as periodic eclosion and cycles in the male courtship song. A polyclonal antibody directed against the *per* gene product stains a few lateral neurons, putative glial cells in many ganglia of the central nervous system, and nuclei of adult photoreceptors. So far no results independent from those derived from '*per*-staining' have been obtained concerning putative sites for pacemaker neurons in fruitflies. Yet, the anti-*per* antibody stains nervous tissues in marine snails and rats, including regions previously characterized as sites of pacemaker neurons. This suggests that the antibody is indeed labelling putative pacemaker sites and that the region of the *per* protein used for developing the antibody might not only be highly conserved in several *Drosophila* species, but also may have some features in common with *per*-like proteins in other animals (see Rosbash and Hall (1989) Neuron 3: 387 for discussion).

In the beetle *Pachymorpha sexguttata* a circadian rhythm of the light sensitivity of the eye has been found. The transmission of the circadian signal to the eyes is fiberbound; intact optic lobes, especially the lobula, are necessary to sustain this rhythm (Fleissner (1982) J. Comp. Physiol. 149: 311).

Using the anti-*per* antibody we have now observed staining in the dorsal brain as well as in the optic lobes and the suboesophageal ganglion of *Pachymorpha*, which was abolished by preabsorption of the antibody with the *per*- derived peptide used to generate the antibody. In the brain about 20 cells, including their processes, were stained in the dorsally located pars intercerebralis and in a few neurons lateral to the pars intercerebralis. A fibertract could be traced in the suboesophageal ganglion. We also found *per*-like immunoreactivity in all three visual ganglia. A cluster of neurons close to the medulla and two clusters close to the lamina were stained. Processes of these cells innervate the lamina, medulla, and lobula. In addition stained fibers from the central brain were seen projecting into the optic lobes. In the first optic chiasm, glial cells were labelled by this antibody. Since *per*-like staining was found in the 'pacemaker tissues' subserving the ERG rhythm, and yet was also seen within the central brain, it will be interesting to determine whether there could be communication between labelled cells in the various ganglia, or if the CNS stained cells might influence the beetle's locomotor activity rhythms, or both.

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"PER"-REACTIVE NEURONS IN THE BRAIN OF THE SCORPION ANDROCTONUS AUSTRALIS

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Since in Drosophila mutants the per-gene has been found to influence the period length of the circadian locomotor rhythm, immunocytology against the per-product is supposed to mark sites of the circadian oscillator. Results supporting this view are reported e.g. from the hamster and a marine mollusc. But additional stainings of e.g.neuroglia and some epithelia gave rise to the hypothesis that the per-product may only influence coupling.

In the scorpion circadian system neuronal elements (ENSF) which are most likely to function as "system-bus" in the circadian clock system (coupling pathways between different effector systems and the left and right pacemaker) are known down to their synaptic connections. By means of immunocytology a series of different neuronal elements could be traced. A number of neurobiological details is analysed. Therefore per-staining (polyclonal antibody) was tested with this well known system.

The per-immunocytology revealed very few neuronal elements and somata which resemble in their central course and location of the somata the octopaminergic ENSF. But double staining (backfilling of the ENSF with Lucifer Yellow plus per-staining) reveal that there are at least two distinct populations of this type of fibers: while the ENSF itself shows little per-reactivity, an additional fiber tract in close parallelity to the ENSF can be seen as intensively reacting to per-product antibodies.

These anatomical findings in the scorpion brain in combination with the results in the beetle (1) are basis for analysing the physiological meaning of the per-product and for a contribution to the discussion: per as an "oscillator and/or coupling"-marker.

(1) Brandes-Frisch et al. SRBR-Meeting 1990 (Poster-Session)

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REVERSIBLE TRANSCRIPTION INHIBITOR ALTERS PERIOD AND PHASE OF A CIRCADIAN RHYTHM AND BLOCKS SOME EFFECTS OF LIGHT ON PROTEINS. S. Ramasubban and A. Eskin, Dept. of Biochem. and Biophys. Sci., Univ. Houston, Houston, TX 77204.

Interpretation of previous research on the role of transcription in circadian oscillators was difficult because the inhibitors abolished the rhythms. We investigated the role of transcription in the *Aplysia* eye circadian rhythm using a reversible transcription inhibitor, 5,6-dichloro-1-β-d-ribobenzimidazole (DRB). DRB inhibits the synthesis of heterogenous nuclear RNA at the level of initiation. ^{3}H-uridine incorporation into the eye was inhibited about 90% by 10^{-4} M DRB. Recovery after 2 h DRB appeared complete within 1 to 3 hrs following the treatment. DRB appeared to have no effect on translation as measured by ^{3}H-leucine incorporation during a 2 h DRB treatment. DRB (2 h, 10^{-4} M) produced large phase shifts in the circadian rhythm at some phases (3.7 h delay, CT 6-8, N=3) and no phase shifts at other phases. The DRB sensitive phases of the rhythm appear to be between CT 0 and CT14. Continuous DRB treatments (10^{-6}M) altered the free running period of eye rhythms from around 23.5 h to 25.5 h (N=4). The results with DRB indicate that brief disruptions of transcription lead to perturbations of the circadian oscillator. This data suggests, for the first time, that transcription is an important process for the oscillating mechanism. Moreover, DRB will be a valuable tool for exploring the role of transcription in circadian timing.

Another way to study the role of transcription is to investigate if the effects of entraining agents on the rhythm are altered by transcription inhibitors and to correlate this study with one on the role of transcription in mediating effects of light and 5-HT on proteins. As a first step, we investigated the role of transcription in effects of light on proteins. In earlier experiments, we found that light from CT 18-24 changed amino acid incorporation of 7 proteins. DRB appeared to inhibit the effect of light on 5 proteins. These results indicate that transcription is involved in mediating the effect of light on these 5 proteins. The properties of these 5 proteins indicate that they may be components of the entrainment pathway or the oscillator mechanism. Thus, our results with DRB and light suggest that transcription may be involved in the entrainment pathway or the oscillating mechanism. The apparent involvement of different mechanisms mediating the effects of light on different proteins (DRB only blocked the effects of light on 5 of 7 light sensitive proteins) should help us clarify the roles of the proteins. Furthermore, these results demonstrate that it will be important to investigate the effect of light on levels of specific mRNAs. (Supported by MH41979)

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AMINO ACID SEQUENCE AND FUNCTION OF A PUTATIVE CIRCADIAN OSCILLATOR PROTEIN. U. Raju, M. Nunez-Regueiro, *R.Cook, and A. Eskin, Dept. of Biochem. and Biophys. Sci., Univ. of Houston, *Baylor College of Medicine, Houston, TX 77204.

Elucidation of the circadian oscillating mechanism entails identifying its components, determining how the components interact with one another and testing whether these interactions account for properties of the circadian rhythm. The general strategy that we have used to find components of the circadian oscillator is to trace regulatory input pathways to an element of the oscillator.

A reasonable hypothesis is that proteins are components of the regulatory pathways and they also are components of the oscillator. Light and serotonin (5-HT) regulate the circadian rhythm in the eye of *Aplysia*. Therefore, we looked for proteins whose synthesis is modified by light or 5-HT. Using 2D-gel electrophoresis to separate proteins, we found that exposure of eyes to light or 5-HT altered the incorporation of amino acids into a number of proteins. A particularly interesting finding was that a few proteins were modified by both light and 5-HT. As a result of their properties and responses to light and 5-HT, a number of proteins may be considered putative components of circadian oscillators.

As a first step towards determining the role of these proteins in the circadian mechanism we have begun to obtain amino acid sequences of the proteins. Protein spots were cut from 2D-gels, digested with V8 protease, and then separated on a 1D-gel. Peptides were electroblotted from the gel to Immobilon-P membrane and then cut from the membrane and placed into a gas-phase amino acid sequencer. We obtained a 38 amino acid sequence of a peptide derived from one of the most interesting proteins (≈40K, pI 5.6) that we discovered. This protein was affected in opposite ways by both light and 5-HT. Most exciting and important has been the discovery of a significant homology (>60%) between the sequence of the peptide of the 40K protein and published sequences of a family of proteins called annexins. This family of calcium and phospholipid binding proteins are believed to play important roles in cellular regulation. Suggested functions of annexins are inhibition of phospholipase A2 (and thus control of the arachidonic acid second messenger system) and control of membrane protein-cytoskeletal linkages.

The tentative identification of the 40K protein as an annexin has given us new ideas about where to look for the circadian oscillating mechanism. More specific hypotheses can now be tested based on the possible cellular functions of this protein. (Supported by NIMH MH41979)

180 A POSSIBLE MECHANISM OF PERIOD SHORTENING BY CHLORIDE CONDUCTANCE INHIBITION IN THE BULLA OCULAR CIRCADIAN PACEMAKER

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The eye of the marine mollusc Bulla functions as a competent circadian pacemaker in vitro. The retinal pacemaker cells exhibit a rhythm in membrane potential (possibly driven by a rhythmic K^+ conductance) and in compound action potential (CAP) frequency which can be recorded extracellularly from the optic nerve. The period of the pacemaker is lengthened by treatments which chronically change membrane potential, and phase dependent phase shifts are generated by pulse treatments which change membrane potential (light acts by depolarizing). Recent work has demonstrated that treatments which inhibit Cl^- conductance can shorten the period of the rhythm by up to 2.5 hr. per cycle. In this study we address the possible mechanism of this period shortening.

A detailed analysis of the shape and characteristics of the CAP rhythm in Cl^--free artificial seawater (ASW, Cl^- substituted with SO_4) showed that the period shortening was mainly due to a decrease in the length of the non-spiking part of the cycle, which suggested that Cl^- conductance was primarily important during the late subjective night. In order to further examine the phase at which Cl^- conductance inhibition may be acting on the pacemaker, we are constructing a PRC to 6 hour pulses of Cl^--free ASW. Pulses applied at CT 17-23 and CT 14-20 have yielded significant phase advances (+93 min. ±31 C.I., N=8 and +35 min. ±29, N=6 respectively). Pulses applied at CT 8-14, 11-17, and 20-2 did not yield significant phase shifts; the phase shifts were highly variable ranging from phase delays to advances although the majority of shifts in each case were advances. The appearance of phase advances to Cl^- conductance inhibition in the late subjective night is consistent with depolarization-induced phase shifting; light applied at CT 20-23 generates a significant phase advance (+60 min. ±23, N=6). However, if Cl^--free ASW is depolarizing at all phases, one would expect phase delays in the early subjective night (CT 11-17 and possibly CT 8-14) and a lengthening of period with chronic application as has been reported for depolarizing High K^+ treatment. On the other hand a periodic Cl^- conductance, which is present only during the late subjective night could satisfactorily account for these data. We are currently attempting to record intracellular membrane potential in the presence of Cl^--free ASW over various phases and to block the phase advance at CT 17-23 with a hyperpolarizing treatment to evaluate the possible contribution of membrane potential changes in Cl^--free ASW treatments.

181 CIRCADIAN VARIATIONS OF MESSENGER RIBONUCLEIC ACID FOR PEPTIDE SOMATOSTATIN IN THE SUPRACHIASMATIC NUCLEUS. Shin-Ichi T. Inouye, Hiroaki Nagasaki, Junichi Takeuchi and Ako Tokumasu. Lab. Neurophysiology, Mitsubishi Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, JAPAN.

Since the discovery of the Suprachiasmatic Nucleus (SCN) of the hypothalamus as the biological clock, the molecular mechanism of circadian rhythm generation within the SCN are the subject of intense researches. In order to test a hypothesis that transcriptional regulation of the gene is involved in circadian rhythm generation in the SCN, we have performed Northern analysis of the RNA extracted from the SCN tissue with the use of synthesized oligonucleotide probes for the peptide somatostatin. Rats had been adapted to 12:12 LD cycles at least for 2 weeks and then blinded by orbital enucleation at a time 6-10 hrs before tissue dissection. Total RNA samples were extracted by standard phenol-chloroform procedures in the presence of Urea. Tissues from 3-5 rats at the same circadian time yielded 25-40μg total RNA, which was fractionated on the Agarose gel and blotted on the nitrocellulose filter. Probes complementary to a part of somatostatin mRNA were synthesized and radiolabeled by the 3' end tailing procedures. Autoradiographs after the probes were hybridized with the fractionated RNA showed specific bands to the peptide. In particular we found that somatostatin mRNA have circadian variations both in its abundance and molecular length, which is similar for vasopressin. The somatostatin band was most densely stained at around CT0. A second hybridized RNA species with a shorter molecular length was observed only in the SCN of the brain at late subjective night. This finding underlines a molecular mechanism which brings up circadian rhythms in mRNA common to somatostatin and vasopressin.

182 LIGHT-INDUCED C-FOS EXPRESSION IN THE SCN IS PHASE DEPENDENT
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Exposure of rats (Rea, Brain Res Bull 23:577-581, 1989) or hamsters (Rusak and Robertson, Neurosci. Abst. #201.9, 1989) to brief pulses of light induces c-fos expression in a population of cells in the suprachiasmatic nuclei (SCN), raising the possibility that c-fos expression may represent an early event in the response cascade leading to light-induced alterations in pacemaker activity.

Male, Syrian hamsters were housed individually in cages equipped with running wheels and maintained under constant darkness (DD). After 12 days under DD, all animals displayed stable free-running activity rhythms. On day 13 or 14, groups of hamsters (n=5) received 15 minute pulses of 33 lux of white light at either CT18, CT13 or CT6; times at which light pulses cause either phase advances, phase delays, or no phase alterations of the circadian pacemaker, respectively. Two hours after light exposure the amsters were anesthetized, blindfolded, and perfused with paraformaldehyde/lysine/periodate fixative. Fifty micron-thick sections containing the SCN were prepared and immunostained for c-fos protein (FOS) using antiserum (R1B6) provided by Drs Sharp and Sagar (UCSF & VA Med Ctr).

Light exposure at CT13 or CT18 resulted in the appearance of FOS immunoreactive (FOS-ir) cell nuclei densely distributed throughout the SCN. Although stain intensity was somewhat variable, FOS-ir SCN cells were abundant in all animals exposed to light at these times. In contrast, very few, if any, FOS-ir cells were observed in the SCN of animals exposed to light at CT6. These data indicate that c-fos expression in the SCN occurs in association with light-induced alterations in pacemaker activity. (Supported by AFOSR 23126)

183 REGULATION OF THE LENGTH OF THE CELL CYCLE AND ITS PHASES BY THE LIGHT/DARK SCHEDULE. M.L. Wright, S.Jorey, L. Blanchard, L. Garatti, and S.M. Mayrand. College of Our Lady of the Elms, Chicopee, MA.

The cell cycle was studied in the epidermis of the developing hindlimb of late premetamorphic tadpoles of the frog, Rana pipiens. After 3 weeks of acclimation to the light/dark (LD) schedule, groups of tadpoles were injected with ^{3}H-thymidine, and 2 or 3 from the group were sacrificed at intervals thereafter up to 60 or 65 hr. Using autoradiography, fraction of labeled mitoses curves were obtained for 3 cell cycle determinations on each LD schedule, starting at 0, 8, and 16 hr for the 24 hr schedules, and at 0, 10, and 20 hr for the 30 hr LD regimens. Using standard methods for evaluating fraction of labeled mitoses curves, the length of the cell cycle and its phases was determined on circadian 6L:18D and 18L:6D, and noncircadian 12L:18D and 15L:15D, schedules. The findings were also compared to published data from our lab of the cell cycle on 12L:12D.

The cell cycle varied within and among LD schedules in length (T), the duration of the DNA synthetic (S) phase, and the pre- ($G_1+\frac{1}{2}M$) and post- ($G_2+\frac{1}{2}M$) synthetic gaps, which included mitotic time. There was a pattern of 1 short and 2 long cycles on all LD's except 15:15, where there were 1 long and 2 short ones. On all regimens but 6L:18D, where S was very long, the mean S calculated from the 3 cell cycle determinations approximately equalled the length of the LD schedule (24 or 30 hr). T was longer where L was less than D in the schedule, and shorter where L was equal to, or greater than, D. The duration of $G_2+\frac{1}{2}M$ increased as L lengthened on both 24 and 30 hr schedules. S occupied less time in the cycle and $G_1+\frac{1}{2}M$ was longer on schedules such as 18L:6D and 12L:18D, which produced faster tadpole metamorphosis as determined by prior work, whereas slower development was linked with the reverse. The findings indicate that even small changes in the LD regimen produce major changes in the cell cycle, and suggest that cell cycle modulation may be involved in the regulation of tadpole development rate by the LD schedule.

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